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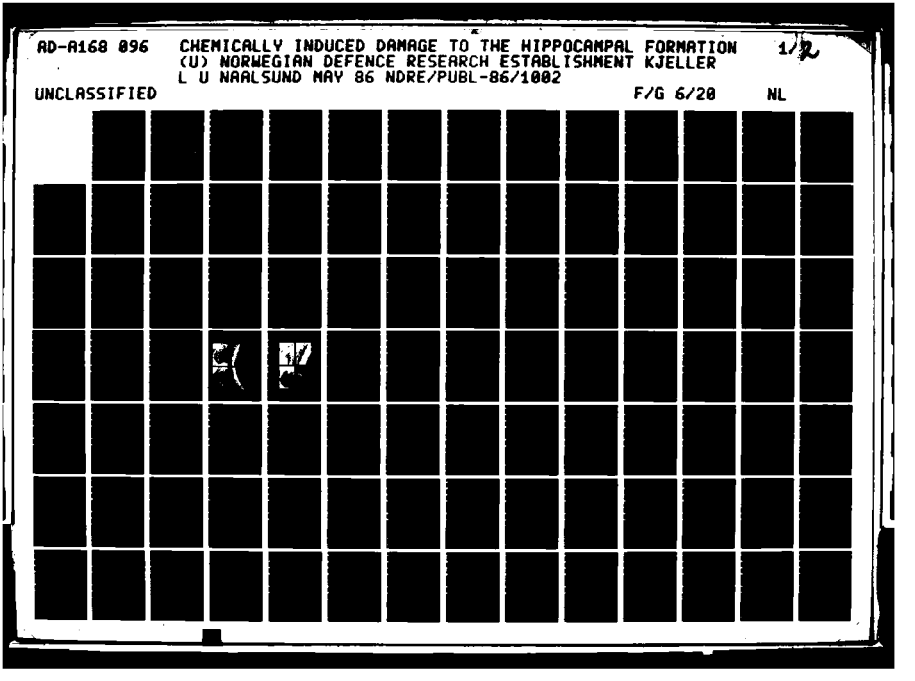

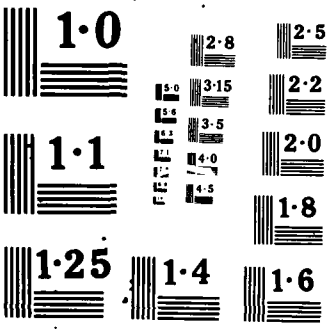

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BY

LIV UNNI NAALSUND

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In the present work it was shown that degeneration of the hippocampal formation after trimethyltin (TMT) administration can be detected at an early phase by eletric recordings from intrahippocampal electrodes. The anatomical distribution of the damage can be studied by histochemistry, and was shown to include the CA3 and CA4 subfields in the initial period and spreading to the CA1 after a complete destruction of the pyramidal cells in CA3 and CA4. The degeneration was shown to be specific for glutamergic neurons in the hippocampus, high affinity uptake of [^{3}H] glutamate being the only biochemical parameter which was changed. *In vitro* TMT increased the efflux of [^{3}H] glutamate from synaptosomes in the resting state, but had no effect on the efflux of [^{3}H] GABA. The synaptosomal reuptake of transmitter and receptor binding were both inhibited in the presence of TMT. In relation to reports on TMT as an

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ABSTRACT (continued)
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PREFACE

The present work has been carried out at the Norwegian Defence Research Establishment (NDRE), Division for Environmental Toxicology, Kjeller, during the years 1982 to 1986. It represents a part of the research program "Neurotoxicity of chemicals in the environment" supported by The Royal Norwegian Council for Scientific and Industrial Research 1984-1986.

I would like to express my sincere gratitude to Professor Dr Philos Frode Fonnum, Head of the Division for Environmental Toxicology, NDRE, and to Dr Philos Reidar Bredo Sund in the Departement of Pharmacology, Institute of Pharmacy, University of Oslo for excellent supervision and continous support during my work. Dr Frode Fonnum and Dr Reidar Bredo Sund constituted the advisory group for this thesis.

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CONTENTS

		Page
1	INTRODUCTION	9
1.1	Hippocampal function	9
1.2	Hippocampal anatomy	10
1.3	Neurotransmitters in the hippocampus	13
1.4	Toxic damage to the hippocampal formation	14
1.5	The aim of the study	15
2	RESULTS AND DISCUSSION	15
2.1	Trimethyltin	15
2.1.1	Neurochemistry	16
2.1.2	Histology	17
2.1.3	Neurophysiology	17
2.1.4	In vitro measurements of chemical transmission	19
2.2	Toluene	21
3	*CONCLUSIONS*	23
	References	23
	Original Papers	37

The thesis is based on the following publications, which will be referred to in the text by Roman numerals:

PAPER I: Naalsund, L U, Allen, C N and Fonnum, F, 1985, Changes in neurobiological parameters in the hippocampus after exposure to trimethyltin, Neuro Toxicology 6(3), 145-58.

PAPER II: (manuscript)
Naalsund, L U and Fonnum, F, 1986, The effect of trimethyltin on three glutamergic and GABAergic transmitter parameters in vitro: high affinity uptake, release and receptor binding, Neuro Toxicology (in press).

PAPER III: (manuscript)
Naalsund, L U and Fonnum, F, 1986, Differences in anionic dependence of the synaptic efflux of D-aspartic acid and γ-amino butyric acid, J Neurochem (in press).

PAPER IV: (manuscript)
Naalsund, L U, 1986, Hippocampal EEG in rats after chronic toluene inhalation, Acta Pharmacol et Toxicol (submitted).

CHEMICALLY INDUCED DAMAGE TO THE HIPPOCAMPAL FORMATION

SUMMARY

In the present work it was shown that degeneration of the hippocampal formation after trimethyltin (TMT) administration can be detected at an early phase by recordings from electrodes implanted in the hippocampal formation. The distribution of the damage can be studied by histology, and was shown to include the pyramidal cells in the CA3 and CA4 subfields in the initial period and spreading to the CA1 after a complete destruction of the pyramidal cells in CA3 and CA4. Neurochemical methods showed that the degeneration was spesific for glutamergic neurons in the hippocampus, and not affecting gabaergic or cholinergic neurons. High affinity uptake of [^{3}H] glutamate was the only biochemical parameter investigated which was changed. In vitro low concentrations of TMT increased the efflux of [^{3}H] glutamate from synaptosomes in the resting state, but had no effect on the efflux of [^{3}H] GABA. The synaptosomal reuptake of transmitter and receptor binding were both inhibited in the presence of TMT. In relation to reports on TMT as an anion conductor, the effect of changes in extracellular Cl^- concentration on transmitter release was investigated. A reduction in the external Cl^- concentration was shown to have a much larger effect on the efflux of transmitter from glutamergic than from GABAergic synaptosomes. The sensitivity of the hippocampal electric activity to changes after exposure to toxic compounds was applied for the investigation of a putative neurotoxic effect of long term exposure to low levels of toluene. An increased synchronization together with an increased frequency in the theta waves was observed during the first period of toluene exposure. The later phase was charachterized by a gradually increasing disruption in regular waves and a reduction in the theta frequency. Small improvements regarding regularity, but no increase in frequency was seen during one month after termination of exposure, indicating that irreversible changes in neuronal functions have occured.

1 INTRODUCTION

1.1 Hippocampal function

The hippocampal formation is one of the most extensively studied areas of the brain. The first theory of the hippocampus as an important olfactory centre, based on its anatomical location in the rhinencephalon or "smell brain" (Ramon y Cajal, 1903; Brodal, 1947), was

displaced by a hypothesis suggesting that the hippocampus is a part of a circuit forming the neural substrate of emotional behavior proposed by Papez in 1937. This hypothesis was extended by MacLean (1952) who introduced the term limbic system, which included the cerebral cortex surrounding the medial part of the cerebral hemisphere and the subcortical nuclei to which it is connected, and suggested that these areas are related to non-visceral functions. Since the introduction of the term limbic system, many brain regions have been shown to have connections with the limbic cortex, and it is now clear that essentially all of the major functional systems in the brain share direct connections with the limbic lobe (Brodal, 1981). This has made it particularly difficult to define the limits of the limbic system, and the old assumption upon which the limbic system concept was based, namely that visceral and non-visceral systems in the brain can be clearly distinguished, seems to be disproved.

Present knowledge suggests that the hippocampal formation may be responsible for intergration of information from the sensory modalities, and transformation of these informations to complex association areas and visceral and motoric control systems. In the broadest functional terms it seems to be in the unique position of influencing somatomotor, visceral, motivational and affective and cognitive mechanisms (Swanson, 1983).

1.2 Hippocampal anatomy

The hippocampal formation has a unique structural organization. The major fiber connections are located in sharply demarcated regions, and cells and fibers form distinct laminae (Andersen et al, 1971). The relative simplicity of its intrinsic neuronal architecture renders the hippocampus a popular model system for the study of basic cell biological functions.

The hippocampal region concists of the area dentata, the hippocampus and the subiculum, see Figure 1.1, (Blackstad, 1956). The principal cells of the area dentata are the granule cells with its excitatory

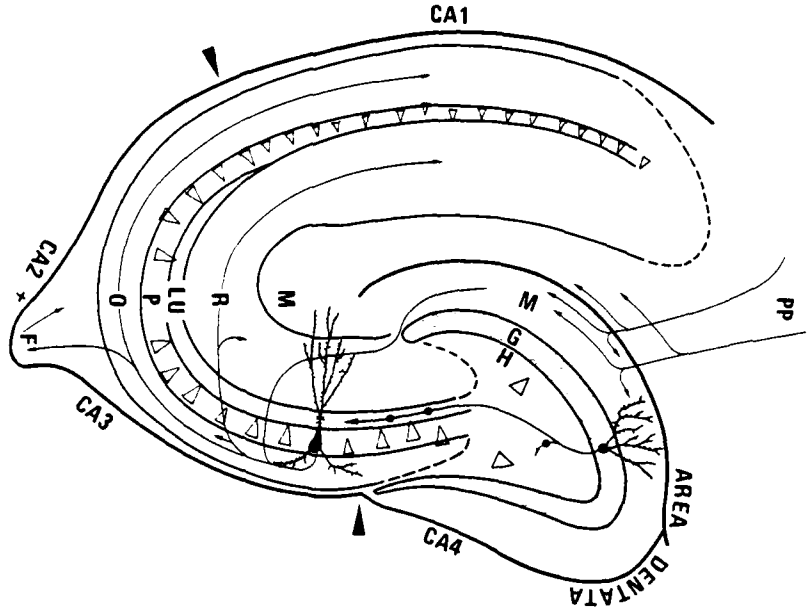

Figure 1.1 Schematic drawing showing the main layers and their connections in the hippocampal formation
Labelling: Hippocampus: F=fimbria, O=stratum oriens, P=stratum pyramidale (with the indicated subfields CA1-4), R=stratum radiatum, M=stratum lacunosum moleculare, LU=stratum lucidum (mossy fibre layer). Area dentata: H=hilus fascia dentata, G=stratum granulosum, M=stratum moleculare. PP=perforant path axons from the enthorhinal cortex distributing to the molecular layer of area dentata. Granular cells give rise to the mossy fibres, which terminates in the hilus of fascia dentata and in stratum lucidum of CA3 and CA4

axons, the mossy fibers, (Blackstad et al, 1970). The pyramidal cells are the major neurons of the hippocampus. They are arranged in a dense, continous layer, the stratum pyramidale, and their dendrites are located in the stratum oriens, stratum radiatum and the stratum lacunosum moleculare (Andersen et al, 1971). The hippocampus is

divided into the subfields CA1-4 (Lorente de No, 1934), in the rat the CA2 is usually included in the CA3 (Blackstad, 1956).

The main inputs to the hippocampus are the perforant path (Lorento de No, 1934; Hjort-Simonsen, 1972), which originates in the entorhinal cortex and terminates in the area dentata, and the septo-hippocampal projection, which originates in the medial septal nucleus and distributes to the whole hippocampal formation and the adjacent cortical areas (Swanson and Cowan, 1979). Impulses entering the granule cells through the perforant path are further processed through the hippocampal formation by a three-membered neuronal chain, i e the mossy fibers, the CA3 axons and the CA1 axons. The CA3 neurons receive impulses from the mossy fibers (Blackstad et al, 1970) and propagate these impulses through the Schaffer collaterals to the ipsilateral CA1 region (Schaffer, 1892) and through the commisural fibers to the contralateral CA1 and CA3 region (Blackstad, 1956; Laurberg, 1979). The excitatory CA1 pyramidal cells send most of their axons caudally into the subicular complex (Andersen et al, 1973). All these excitatory pathways appear to be monosynaptically conected (Andersen, 1975), but they are also influenced by recurrent inhibition at each step (Andersen et al, 1964). Local interneurons are present both in the area dentata and in the stratum pyramidale of the hippocampus (Storm-Mathisen, 1972; Fonnum and Walaas, 1978).

The septo-hippocampal connections are mediated through the fimbria and fornix. Both CA1 and CA3 pyramidal cells projects to the lateral septum via fimbria (Swanson and Cowan, 1977). The lateral septum projects to the medial septum, which in turn projects back to the hippocampal subfields as well as to the dentate granular cells and the subicular complex (Swanson, 1978; Swanson and Cowan, 1979).

The summarized connections represents the quanitatively important part of the hippocampal circuitry. More detailed descriptions are given in reviews by Andersen, 1975, and Walaas, 1983.

1.3 Neurotransmitters in the hippocampus

The unique anatomy of the hippocampal formation, decribed above has made it an attractive brain area for neurotransmitter studies. The acidic amino acids, glutamate and aspartate are probably the most important neurotransmitters in the hippocampus. The entorhinal perforant path is a well documented glutamergic or aspartergic pathway (Nadler et al, 1976; 1978; Cotman and Nadler, 1981; Storm-Mathisen, 1977; 1981), while the role of acidic amino acids in the hippocampal mossy fibers is yet uncertain. Some evidence do however exist for this fibers utilizing glutamate or aspastate as well (Storm-Mathisen and Iversen, 1979; Aamodt et al, 1984). Both the CA3 and CA1 excitatory fibers may also utilize glutamate or aspartate as transmitter (Nadler et al, 1976; Storm-Mathisen, 1977; 1978; Fonnum and Walaas, 1978; Fonnum et al, 1979).

Acetylcholine is the excitatory transmitter of about 50% of the afferents from medial septum entering the hippocampus through fimbria (Lewis and Shute, 1967; Lewis et al, 1967; Storm-Mathisen, 1972; Wainer et al, 1985). The transmitter of the non-cholinergic fimbrial fibers are at present not identified. GABA neurons in the hippocampal formation are mainly local neurons (Fonnum and Storm-Mathisen, 1969; Storm-Mathisen and Fonnum, 1971) probably involved in both recurrent inhibition of the principal projection neurons in the region and regulation of local excitability in the dendritic tree that receives the major excitatory inputs (Storm-Mathisen and Fonnum, 1971). A serotonergic input, mainly derived from median raphe nucleus (Lorens and Guldberg, 1974) excerts an inhibitory action on the hippocampus (Stefanis, 1964; 1968). The catecholamine level in the hippocampus is low and is mainly located in noradrenergic fibers, which originate in the brainstem, and releases noradrenaline excerting a depressant action on the pyramidal cells (Segal and Bloom, 1974; 1976). The dopamine content of the hippocampus is less than 5% of the noradrenaline level (Bischoff et al, 1979). A restricted dopamine input arising in the substantia nigra (Swanson, 1982) to the hippocampus and area dentata is however believed to exist (Scatton, 1980).

1.4 Toxic damage to the hippocampal formation

Hippocampus appears to be extremely sensitive to the action of several neurotoxic compounds such as kainic acid (Olney et al, 1974), 3-acetylpyridin (Cogeshall and Mac Lean, 1958), glucocorticoids (Sapolsky, 1985), trimethyltin (Brown et al, 1979) as well as to cell loss induced by ischemia (Kirino, 1984), hypoglycemia (Auer et al, 1984), seizures (Folbergrova et al, 1981) and Alzheimers disease (Henke and Lang, 1983).

The selective vulnerability of the hippocampus was recognized early in the history of neuroscience, and the differences in vulnerability of the hippocampal subfields has been well described (Friede, 1966). However, so far it has not been possible to draw any conclusions about the mechanisms behind selective vulnerability of neuronal tissue. As no correlation has been demonstrated between regional cerebral blood flows and regional neuronal necrosis (Wieloch, 1985) the vulnerable neurons are thought to be subject to local stress, or to posess particular properties making them prone to insults. The pyramidal cells in the hippocampus and cortical regions as well as the cerebellar Purkinje cells are thought to possess a high vulnerability to anoxic damage due to their large soma and high metabolic activity (Norton, 1980). A striking common property of the brain areas selectively vulnerable to an ischemic insult, CA1 and subicular region of the hippocampus, hilus of area dentata, lateral septum,the amygdaloid nuclei, entorhinal cortex, the olfactory tubercle and the reticular nucleus of the thalamus (Wieloch, 1985), is that they all receive a dense excitatory aminoacidergic innervation (Foster and Fagg, 1984, Wieloch, 1985). The density and subtype distribution of excitatory amino acid receptors on postsynaptic membranes have been shown to posess patterns related to selective vulnerability (Monaghan et al, 1983). The intracellular second messenger used by the excitatory amino acids is not yet identified (Wieloch, 1985), however ionic movements over the plasma membrane have been suggested to be possible mediators of the neurotoxic actions of these substances. Hypotheses regarding such phenomena include either an excessive influx of calsium (Siesjø, 1981) or an enhanced chloride influx into cells (Wieloch, 1985).

1.5 The aim of the study

The aim of the present study was to charachterize the development of the toxic damage in hippocampus induced by trimethyltin. Previous studies have shown that trimethyltin may produce selective damage to the hippocampal pyramidal cells (Brown et al, 1979). It was therefore interesting to study this damage as a model for neurotoxic degeneration in the hippocampus. By using neurochemical, histochemical and neurophysiological tecniques we wanted to compare the time course of changes in the different parameters and then study in detail the effect of this compound on the biochemical events of synaptic transmission. Based on the results from these experiments we chose to study neurophysiological changes in the hippocampus in an investigation of a possible neurotoxic action of the organic solvent toluene.

2 RESULTS AND DISCUSSION

2.1 Trimethyltin

Trimethyltin induced hippocampal damage was detected by neurochemical, histochemical and neurophysiological methods at different developmental stages of the degenerative process (paper I). In vitro studies showed that the neurotransmitter release, high affinity uptake and receptorbinding are interupted at low concentrations of trimethyltin (paper II). The effect on release was quantitatively different for GABA and L-glutamate (paper II). A similar quantitative difference between the release of GABA and D-aspartate was observed when varying concentrations of Cl^- was included in the perfusion buffer (paper III).

2.1.1 Neurochemistry

Neurochemical markers spesific for a certain cell population is frequently chosen as a measure for the number of actively functioning cells of the population under study in a brain region. Glutamate decarboxylase (GAD) is located in the nerve endings of GABAergic neurons while choline acetyltransferase is located in the cholinergic nerve endings. As there is no enzyme spesific for glutamergic terminals, the high affinity uptake of glutamate, spesifically present in glutamergic synaptic terminals, is used as marker for glutamergic neurons. A reduction in the activity of the markers is seen when neurons are completely degenerated (Fonnum, 1977).

The injection of TMT in rats produced a gradually increasing loss of activity of the high affinity uptake of glutamate in the hippocampal CA1 region (paper I). Five weeks after the first of three weekly injections of 3 mg/kg TMT, a 68% reduction in high affinity uptake of glutamate was found. The first significant change was detected four weeks after the first injection (paper I). These results indicate that a complete degeneration of a fraction of the glutamergic nerve terminals was obtained after four weeks. A single large dose of TMT (8 mg/kg) produced a significant loss of glutamergic neurons (33%) seven days after the injection and increasing to a 42% reduction after 3 weeks (paper I), suggesting a faster destruction process after a single, large dose of TMT than after several smaller doses.

The GABAergic and cholinergic markers were not reduced after the TMT treatment of the rats (paper I). A selective destruction of glutamergic neurons seems to take place. Previous studies have reported changes also in the GABAergic markers, a reduced concentration of GABA within the hippocampus (Mailman et al, 1983) and a reduced high affinity uptake of $[^3H]$ GABA has been reported (Doctor et al, 1982). The reduced uptake of GABA was detected 2 hours following administration of 4 mg/kg TMT i p (Doctor et al, 1982) suggesting a direct effect on the active transport rather than a cellular degeneration of the basket cells utilizing GABA as their transmitter. The cholinergic activity was increased five weeks after the multiple injection schedule

(paper I). This may represent sprouting of surviving axons, previously described after lesions in the area dentata (Matthews et al, 1976) and in the hippocampus (Gage et al, 1983; Dravid and Van Deusen, 1984). The phenomenon termed reactive synaptogenesis in many cases represents heterotypic reconstruction not associated with functional recovery (Mc Couch et al, 1958).

2.1.2 Histology

Histological examinations of the brains of TMT intoxicated rats show that the CA4 and ventral CA3 pyramidal cells are the most vulnerable to toxic damage produced by TMT (paper I; Brown et al, 1979; Chang and Dyer, 1983; Valdes et al, 1983). The CA1 pyramidal cells are less sensitive to TMT induced degeneration, they do not disappear completely during the time course of our study as do the CA3 and CA4 pyramidal cells. Differences in vulnerability of the hippocampal subfields has been described in several instances (Friede, 1966) and has been proposed to be due to differences in their chemical composition (Friede, 1966). A sharp chemical border exists between CA1 and CA2-3 (Friede, 1966), corresponding well with their different reaction to toxic damage.

Degenerating neurons are found in the CA3 and CA4 subfields 21 days after the first of three weekly injections of TMT (3 mg/kg) (paper I). At this time no change is found in the biochemical parameters. Similarly after a single injection of TMT (8 mg/kg), degenerating neurons are found in CA1, CA3 and CA4 hippocampal subfields three days post injection, that is four days prior to any reduction in high affinity uptake of glutamate (paper I).

2.1.3 Neurophysiology

The electrical activity in the hippocampus is a well described characteristic of this region, consisting of two distinguishable wave patterns closely related to the behavior of the rat. The electrographic record of a motionless rat consists of low frequency asynchronous

waves and includes variably occuring large amplitude waves (Vanderwolf, 1969). This EEG pattern is referred to as irregular activity. Rhytmic slow activity (RSA) accompanies voluntary movements such as walking, rearing and mooving the body. RSA is typically regular synchronous electrical waves with a frequency of 7-8 Hz (theta-activity) in the walking rat (Vanderwolf, 1969). A reduction in the frequency of the theta activity occures two weeks after the first of three weekly injections of TMT (3 mg/kg), and further frequency reduction occures between day 14 and day 21 together with a total loss of regular waves (paper I). A single dose of TMT (8 mg/kg) produces a reduction in the theta frequency three days after administration of the TMT (paper I; Ray, 1981). Both amplitude, frequency and incidence of theta are reduced. A total loss of regular waves occures during the third week after a single TMT injection (paper I). Ray (1981) observed that the decreased theta was preceded by an initial period of increased incidence and increased frequency of the theta activity and a presence of theta waves in the immobile rats. Human intoxications with organic tin compounds also produces general disturbance and dysrhytmia of the EEG-recording (Prüll and Rompel, 1970).

The hippocampal response to evoked potentials increases during the first 2-4 days after TMT administration and falls gradually from day 4-6 onward (Ray, 1981; Hasan et al, 1982). In the initial period of TMT intoxication an increased vulnerability to production of seizures is also seen (Dyer et al, 1982b). A phase of increased neuronal excitability is suggested to precede neuronal damage (Ray, 1981). The increased activity of the pyramidal cells may also stem from an increased activity of the mossy fibers due to a reduction in the inhibitory activity of the basket cells on the granule cells, showed by Dyer and Boyes (1984). The possibilty of increased occurence of seizures as the cause of neuronal damage in the hippocampal formation was disproved by Zimmer et al (1985), showing that continously treatment with an anticonvulsant agent did not affect the pathological changes after TMT treatment. Doctor and Fox (1982) even found a reduced responsiveness to electroshock seizures following i p injection of 0.7 and 3.5 mg/kg TMT to mice. These results indicate that the neuronal mechanisms of action of TMT differs from the action

of kainic acid, a neurotoxic compound with a similar selective neuronal damage as TMT. Kainic acid is dependent upon seizure activity for production of pathological changes in the hippocampus (Ben-Ari et al, 1980). In contrast to the observations on increased excitability of the hippocampus, TMT has a depressive effect on hippocampal pyramidal cells in vitro, suggesting a decreased excitability of the postsynaptic neuron, or a decreased release of transmitter during stimulation of the pyramidal neuron (Allen and Fonnum, 1984).

2.1.4 In vitro measurements of chemical transmission

Several neurochemical mechanisms may be involved in the observed neuronal changes during TMT intoxication. In vitro alkyltin compounds causes a disruption of mitochondrial function (Aldridge, 1958; Selwyn, 1976) including an inhibition of oxidative phosphorylation (Selwyn, 1976). The ability of neuronal cells to perform chemical transmission makes them vulnerable to any change in biochemical activity. Both the release, high affinity uptake and spesific receptor binding of the neurotransmitter is affected by TMT at different concentration levels. The release of L-[^{3}H] glutamic acid and D-[^{3}H] aspartic acid from synaptosomes in vitro is enhanced by 20% at TMT concentrations from 50 µM to 1 mM (paper II). At the 50 µM concentration level a 25 - 30% reduction in the high affinity uptake (paper II) and in spesific receptorbinding of glutamate (paper II) is observed. The release of [^{3}H] GABA is unaffected by the presence of TMT at concentrations up to 1 mM (paper II), while the high affinity uptake of [^{3}H] GABA is reduced at the highest TMT concentrations (0.5 - 1 mM) and receptor binding at 50 nm ligand concentration is inhibited with 100 µM or more TMT in the medium. The lowest concentration of TMT which interupts the neurotransmission, 50 µM (= 50 nmol/ml), is about five times the in vivo concentration after injection of a neurotoxic dose of TMT. Brown et al (1979) reports that the minium amount of TMT in brain tissue to cause neuronal necrosis is 8.6 nmol/g wet wt.

The TMT stimulated release of glutamate is much smaller but has the same characteristics of the K^+ stimulated release in that it is Ca^{++} dependent and TTX independent (paper II). The effect of TMT on gluta-

mate release is quite different from the effect on ACh release, which is inhibited during depolarization but unaffected at low K^+ concentrations (5 mM) (Morot-Gaudry, 1984) in the presence of high concentrations of TMT (0.5 - 3.5 mM). Morot-Gaudry showed that the alkyltin was unable to inhibit transmitter release induced by a calcium ionophore, and suggested that the action of TMT is an interference with calcium conductance. The effect on ACh transmission could also be secondary to an inhibition of mitochondrial function (Aldridge, 1958; Selwyn, 1976), since the synthesis of ACh in the cytosol is dependent upon acetyl-Co A formed in the mithochondria (Quastel, 1977).

Organotin compounds have been shown to induce several effects on membrane systems. They are associated with Cl^-/OH^- exchanges in mitochondria (Selwyn et al, 1970), erythrocytes and liposomes (Selwyn et al, 1970; Motais et al, 1977; Wieth and Tosteson, 1979) and in pancreatic β-cells (Pace and Tarvin, 1983). This exchange could possibly explain the effect of TMT on glutamate release through a change in the relation between internal and external Cl^- concentration. A reduction in the extracellular Cl^- concentration induces a glutamate release similar to that induced by high potassium (paper III; Hardy et al, 1984), but do not have the same stimulatory effect on the release of the inhibitory transmitter GABA (paper III). A reduction in the extracellular Cl^- concentration from 145 mM to 125 mM is sufficient to produce a significant release of transmitter from glutamergic and aspartergic neurons (paper III), while the release of GABA is unaffected by the same change in Cl^--concentration.

The putative neurotransmitter amino acids glutamate and aspartate can cause neurotoxic effects when present in excess in nervous tissue (Olney et al, 1971; Olney, 1976). A delicate balance may exist between the function of these substances as excitatory transmitters and their capasity to cause nerve cell death. The increased leakage of tritiated transmitter together with a reduced uptake (paper II) should give an increase in the extracellular level of the neurotransmitter in the synaptic cleft. This effect seems to be spesific for glutamergic and aspartergic neurons at low doses (paper II and III). This is in agreement with Dyer et al (1982a) who suggested that TMT may induce an

excitotoxic action. The reduced post-synaptic activity in the presence of TMT (paper II; Allen and Fonnum, 1984), should however, reduce the effect of an excess glutamate efflux into the synaptic cleft. Another possibility of the site of primary toxic damage is that it occurs pre-synaptically in the terminal region as a result of ionic disturbances and transmitter depletion (paper II; Selwyn, 1970). The resulting degeneration process could be a retrograde degeneration of the axon. Electronmicroscopic examinations of early damage shows degenerating axons in chronically intoxicated rats (Bouldin et al, 1981), but no descriptions on terminal structures have been given in the litterature.

2.2 Toluene

On the background of the results from TMT studies we chose the hippocampal electric activity as the parameter in an investigation of possible neurotoxic effects of the organic solvent toluene. Toluene inhalation produces disruptions and frequency changes in the hippocampal theta activity (paper IV; Takeuchi and Hisanaga, 1977). The time course of changes in the hippocampal electric activity follows the same phases as described for TMT (Ray, 1981; Hasan et al, 1982; paper I). After an initial increase in the frequency and an increase in the occurence of theta waves, there is a reduction in the frequency and a gradually increased disruption of the theta waves (paper IV). High toluene concentrations (4000 ppm) will produce acute frequency reduction in the theta waves, when recorded during and within two hours after exposure (Tackeuchi and Hisanage, 1977).

One month of recovery after the termination of toluene exposure do not allow any improvements to occure in the theta-activity, suggesting that an irreversible brain damage is developed (paper IV). A neuronal damage causing disruption in theta activity could be located in any of the brain nuclei involved in generating or regulating the theta rhytm. The main source for hippocampal desynchronization is thought be the median raphe nucleus (Vertes, 1982), while the medial pontine region of the reticular formation seems to be the source of hippocampal syn-

chronization (Vertes, 1982). Brain stem generation of hippocampal activity is mediated by the medial septum (Vertes, 1982). By use of surgical lesions it has been shown that lesions in the medial septum can give a complete loss of hippocampal rhytmic slow activity (theta) (Andersen et al, 1979), while lesions in the brain stem and pontine area give a reduction in frequency without any qualitative disturbances (Vertes, 1982). The reason for this lack of effect of brain stem lesions in abolishing theta activity is thought to be the diffuse organization of the ascending brain stem systems and the fact that several pathways maintain the theta activity (Vertes, 1982). Studies on pharmacologic manipulations of the theta-rhythm describes acute effects, and our experiments can therefore not be readily compared to any of these studies (Stumpf, 1965). The toluene level in the brain tissue declines with the same rate as the bloodlevel, and 24 hours after an inhalation exposure the brain level is 0.1% of the concentration at the termination of exposure (Pyykkö et al, 1977). We have measured the hippocampal EEG 48 hours after exposure each week and hence no acute effects have been detected.

Toluene produces alterations in CNS excitability (Benignus, 1981), sensory funtion (Pryor et al, 1983a; 1983b; Dyer et al, 1984) and abnormalities in EEG recordings (Takeuchi and Hisanaga, 1977), all of which might be previous effects to the observed total loss of hippocampal rhytmic activity (paper IV). Several cases of abnormal EEG in humans, either occupationally or voluntary exposed to toluene is reported (Andersen and Kaada, 1953; Knox and Nelson, 1966; Seppäläinen et al, 1980; Grasso et al, 1984).

Changes in biochemical parameters related to aminergic and cholinergic neurons occurs in hippocampus (Yamawaki et al, 1982; Honma, 1983), brain stem (Yamawaki et al, 1982; Rea et al, 1984) and hypothalamic nuclei (Andersson et al, 1980) after short term exposure of rats to toluene levels from 500 to 8000 ppm, supporting the suggestion of a neuronal damage in the theta-generating neural axis from the brain stem through midbrain and septal nuclei to the hippocampus.

3 CONCLUSIONS

1) Trimethyltin (TMT) caused a selective neuronal damage to the hippocampal subfields CA3, CA4 and partly to CA1. The cell loss in these areas increased gradually during one month after the first administration of TMT.

2) The hippocampal damage produced by TMT could be detected by either neurophysiological, histological or biochemical methods, the neurophysiological being the most sensitive.

3) Degenerating cells in the hippocampus after TMT-intoxication are glutamergic.

4) TMT induced release of glutamate from resting cells, and inhibited reuptake and receptor binding of this neurotransmitter. Release of GABA was not induced by TMT, however reuptake and receptor binding of GABA were inhibited.

5) The synaptosomal efflux of D-aspartate is much more sensitive than the efflux of GABA to changes in extracellular Cl^- concentrations.

6) Toluene produced irreversible loss of hippocampal theta activity after exposure to 500 ppm of the solvent for 8 hours/day, 5 days/week in 12 weeks.

7) Following toluene exposure an initial period of increased synchronous electric activity in the hippocampus was followed by a gradually increased disruption and a reduction in frequency of the theta waves.

References

Aamodt, A, Aambø, A, Walaas, I, Søreide, A J and Fonnum, F (1984): Autoradiographic demonstration of glutamate structures after stereotaxic injection of kainic acid in rat hippocampus, Brain Res 294, 341-45.

Aldridge, W N (1958): The biochemistry of organotin compounds, Biochem J 69, 367-76.

Allen, C N and Fonnum, F (1984): Trimethyltin inhibits the activity of hippocampal neurons recorded in vitro, NeuroToxicology 5, 23-30.

Andersen, P (1975): Organization of hippocampal neurons and their interconnections. In: The Hippocampus, Isaacson, R L and Pribram, K H eds, pp 155-75, Plenum publ, New York.

Andersen, P and Kaada, B R (1953): The electroencephalogram in poisoning by lacquer thinner (Butyl acetat and toluene), Acta Pharmacol et toxicol 9, 125-30.

Andersen, P, Eccles, J C and Løyning, Y (1964): Pathway of postsynaptic inhibition in the hippocampus, J Neurophysiol 27, 608-19.

Andersen, P, Bliss, T V P and Skrede, K K (1971): Lamellar organization of hippocampal excitatory pathways, Exp Brain Res 13, 222-38.

Andersen, P, Bland, B H and Dudar, J D (1973): Organization of the hippocampal output, Exp Brain Res 17, 152-68.

Andersen, P, Bland, H B, Myhrer, T and Schwartzkroin, P A (1979): Septo-hippocampal pathway necessary for dentate theta production, Brain Res 165, 13-22.

Andersson, K, Fuxe, K, Toftgård, R, Nilsen, O G, Eneroth, P and Gustafsson, J-Å (1980): Toluene-induced activation of certain hypothalamic and median eminence catecholamine nerve terminal systems of the male rat and its effects on anterior pituitary hormone secretion, Toxicol Letts 5, 393-98.

Auer, R N, Wieloch, T, Olsson, Y and Siesjø, B K (1984): The distribution of hypoglycemic brain damage, Acta Neuropathol (Berl) 64, 177-91.

Ben-Ari, Y, Tremblay, E, Ottersen, O P and Meldrum, B S (1980): The role of epileptic activity in hippocampal and "remote" cerebral lesions induced by kainic acid, Brain Res 191, 79-97.

Benignus, V A (1981): Health effects of toluene: A review, NeuroToxicology 2, 567-88.

Bischoff, S, Scatton, B and Korf, J (1979): Biochemical evidence for a transmitter role of dopamine in the rat hippocampus, Brain Res 165, 161-65.

Blackstad, T W (1956): Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination, J Comp Neurol 105, 417-521.

Blackstad, T W, Brink, K, Hem, J and Jeune, B (1970): Distribution of hippocampal mossy fibers in the rat, An experimental study with silver impregnation methods, J Comp Neurol 138, 433-50.

Brodal, A (1947): The hippocampus and the sense of smell, A review, Brain 70, 179-222.

Brodal, A (1981): Neurological Anatomy in Relation to Clinical Medicine, 3rd Edition, Oxford University Press, New York and Oxford.

Brown, A W, Aldridge, W N, Street, B W and Verschoyle, R D (1979): The behavioral and neuropathologic sequelae of intoxication by trimethyltin compounds in the rat, Am J Pathol 97, 59-82.

Bouldin, T W, Goines, N D, Bagnell, C R and Krigman, M R (1981): Pathogenesis of trimethyltin neuronal toxicity, Am J Pathol 104, 237-49.

Chang, L W and Dyer, R S (1983): A time-course study of trimethyltin induced neuropathology in rats, Neurobehav Toxicol Teratol 5, 443-59.

Coggeshall, R E and MacLean, P D (1958): Hippocampal lesions following administration of 3-acetylpyridine, Proc Soc Exp Biol Med 98, 687-89.

Cotman, C W and Nadler, J V (1981): Glutamate and aspartate as hippocampal transmitters: Biochemical and pharmacological evidence. In: Glutamate: Transmitter in the Central Nervous System, Roberts, P J, Storm-Mathisen, J and Johnston, G A R, eds, pp 117-54.

Doctor, S V and Fox, D A (1982): Effects of organotin compounds on maximal electroshock seizure (mes) responsiveness in mice, I, Tri (n-alkyl)tin compounds, J Toxicol Environ Health 10, 43-52.

Doctor, S V, Costa, L G, Kendall, D A and Murphy, S D (1982): Trimethyltin inhibits uptake of neurotransmitters into mouse forebrain synaptosomes, Toxicology 25, 213-21.

Dravid, A R and Van Deusen, E B (1984): Recovery of enzyme markers for cholinergic terminals in septo-temporal regions of the hippocampus following selective fimbrial lesions in adult rats, Brain Res 324, 119-28.

Dyer, R S and Boyes, W K (1984): Trimethyltin reduces recurrent inhibition in rats, Neurobehav Toxicol Teratol 6, 369-71.

Dyer, R S, Walsh, T J, Wonderlin, W F and Bercegeay, M (1982a): The trimethyltin syndrome in rats, Neurobehav Toxicol Teratol 4, 127-33.

Dyer, R S, Wonderlin, W F and Walsh, T J (1982b): Increased seizure susceptibility following trimethyltin administration in rats, Neurobehav Toxicol Teratol 4, 203-8.

Dyer, R S, Muller, K E, Janssen, R, Barton, C N, Boyes, W K and Benignus, V A (1984): Neurophysiological effects of 30 day chronic exposure to toluene in rats, Neurobehav Toxicol Teratol 6, 363-68.

Folbergrovà, J, Ingvar, M and Siesjø, B K (1981): Metabolic changes in cerebral cortex, hippocampus, and cerebellum during sustained bicucullin-induced seizures, J Neurochem 37, 1228-38.

Fonnum, F (1977): Acetylcholine and amino acids; Transmitter candidates in rhinencephalon. In: Bel-Air Symposium, V, pp 61-72, George and Co, Geneva.

Fonnum, F and Storm-Mathisen, J (1969): GABA synthesis in rat hippocampus correlated to the distribution of inhibitory neurones, Acta Physiol Scand 76, 35A-37A.

Fonnum, F and Walaas, I (1978): The effect of intrahippocampal kainic acid injections and surgical lesions on neurotransmitters in hippocampus and septum, J Neurochem 31, 1173-81.

Fonnum, F, Lund Karlsen, R, Malthe-Sørensen, D, Skrede, K K and Walaas, I (1979): Localization of neurotransmitters, particularly glutamate, in hippocampus, septum, nucleus accumbens and superior colliculus, Progr Brain Res 51, 167-91.

Foster, A C and Fagg, G E (1984): Acidic amino acid binding sites in mammalian neuronal membranes: Their characteristics and relationship to synaptic receptors, Brain Res Rev 7, 103-64.

Friede, R L (1966): The histochemical architecture of the ammon's horn as related to its selective vulnerability, Acta Neuropath 6, 1-13.

Gage, F H, Bjørklund, A and Stenevi, U (1983): Reinnervation of the partially deafferented hippocampus by compensatory collateral sprouting from spared cholinergic and noradrenergic afferents, Brain Res 268, 27-37.

Grasso, P, Sharratt, M, Davies, D M and Irvine, D (1984): Neurophysiological and psychological disorders and occupational exposure to organic solvents, Food Chem Toxicol 22, 819-52.

Hardy, J A, Boakes, R J, Thomas, D J E, Kidd, A M, Edwardson, J A, Virmani, M, Turner, J and Dodd, P R (1984): Release of aspartate and glutamate caused by chloride reduction in synaptosomal incubation media, J Neurochem 42, 875-77.

Hasan, Z, Woolley, D E and Zimmer, L (1982): Effects of trimethyltin on potentials evoked in the limbic system of the rat, Proc West Pharmacol Soc 25, 449-52.

Henke, H and Lang, W (1983): Cholinergic enzymes in neocortex, hippocampus and basal forebrain of non-neurological and senile dementia of Alzheimer-type patients, Brain Res 267, 281-91.

Hjort-Simonsen, A (1972): Projection of the lateral part of the entorhinal area to the hippocampus and fascia dentata, J Comp Neurol 146, 219-31.

Honma, T (1983): Changes in acetylcholine metabolism in rat brain after a short-term exposure to toluene and n-hexane, Toxicol Letts 16, 17-22.

Kirino, T and Sano, K (1984): Selective vulnerability in the gerbil hippocampus following transient ischemia, Acta Neuropathol (Berl) 62, 201-8.

Knox, J W and Nelson, J R (1966): Permanent encephalopathy from toluene inhalation, N Eng J Med 275, 1494-96.

Laurberg, S (1979): Commissural and intrinsic connections of the rat hippocampus, J Comp Neurol 184, 685-708.

Lewis, P R and Shute, C C D (1967): The cholinergic limbic system: Projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supraoptic crest, Brain 90, 521-40.

Lewis, P R, Shute, C C D and Silver, A (1967): Confirmation from choline acetylase analyses of a massive cholinergic innervation to the rat hippocampus, J Physiol (Lond) 191, 215-24.

Lorens, S A and Guldberg, H C (1974): Regional 5-hydroxytryptamine following selective midbrain raphe lesions in the rat, Brain Res 78, 45-56.

Lorente de Nó, R (1934): Studies on the structure of the cerebral cortex, II, Continuation of the study of the ammonic system, J Psychol Neurol 46, 113-77.

MacLean, P D (1952): Some psychiatric implications of physiological studies on frontotemporal portion of limbic system (visceral brain), Electroenceph Clin Neurophysiol 4, 407-18.

Mailman, R B, Krigman, M R, Frye, G D and Hanin, I (1983): Effects of postnatal trimethyltin or triethyltin treatment on CNS catecholamine, GABA and acetylcholine systems in the rat, J Neurochem 40, 1423-29.

Matthews, D A, Cotman, C and Lynch, G (1976): An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat, II, Reappearance of morphologically normal synaptic contacts, Brain Res 115, 23-41.

McCouch, G P, Austin, G M, Liu, C N and Liu, C Y (1958): Sprouting as a cause of spacticity, J Neurophysiol 21, 205-16.

Monaghan, D T, Holets, V R, Toy, D W and Cotman, C W (1983): Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding sites, Nature 306, 176-79.

Morot-Gaudry, Y (1984): Inhibition by trimethyl-tin, probenecid and phenylglyoxal of depolarization-induced acetylcholine release in torpedo synaptosomes, Neurochem Int 6, 553-61.

Motais, R, Cousin, J L and Sola, F (1977): The chloride transport induced by trialkyl-tin compound across erythrocyte membrane, Biochim Biophys Acta 467, 357-63.

Nadler, J V, Vaca, K W, White, W F, Lynch, G S and Cotman, C W (1976): Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents, Nature 260, 538-40.

Nadler, J V, White, W F, Vaca, K W, Perry, B W and Cotman, C W (1978): Biochemical correlates of transmission mediated by glutamate and aspartate, J Neurochem 31, 147-55.

Norton, S (1980): Toxic responses of the central nervous system. In: Casarett and Doull's Toxicology, Doull, J, Klaassen, C D and Amdur, M O eds, pp 179-205, Macmillan Publishing Co, New York.

Olney, J W (1976): Brain damage and oral intake of certain amino acids, Adv Exp Med Biol 69, 497-506.

Olney, J W, Ho, O L and Rhee, V (1971): Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system, Exp Brain Res 14, 61-76.

Olney, J W, Rhee, V and Ho, O L (1974): Kainic acid: a powerful neurotoxic analogue of glutamate, Brain Res 77, 507-12.

Pace, C S and Tarvin, J T (1983): pH modulation of glucose-induced electrical activity in B-cells: involvement of Na/H and HCO_3/Cl antiporters, J Membrane Biol 73, 39-49.

Papez, J W (1937): A proposed mechanism of emotion, Arch Neurol Psychiat 38, 725-43.

Prüll, G and Rompel, K (1970): EEG changes in acute poisoning with organic tin compounds, Electroenceph Clin Neurophysiol 29, 215.

Pryor, G T, Dickinson, J, Howd, R A and Rebert, C S (1983a): Neurobehavioral effects of subchronic exposure of weanling rats to toluene or hexane, Neurobehav Toxicol Teratol 5, 47-52.

Pryor, G T, Dickinson, J, Howd, R A and Rebert, C S (1983b): Transient cognitive deficits and high-frequency hearing loss in weanling rats exposed to toluene, Neurobehav Toxicol Teratol 5, 53-7.

Pyykkö, K, Tähti, H and Vapaatalo, H (1977): Toluene concentrations in various tissues of rats after inhalation and oral administration, Arch Toxicol 38, 169-76.

Quastel, J H (1977): Source of the acetyl group in acetylcholine. In: Cholinergic Mechanisms and Psychopharmacology, Jenden, D J, ed, pp 411-30, Plenum Press, New York.

Ramon y Cajal, S (1903): Die riechrinde beim menschen und säugetier, Studien über die hirnrinde des menschen, 4. heft, Verlag von Johann Ambrosius Barth, Leipzig.

Ray, D E (1981): Electroencephalographic and evoked response correlates of trimethyltin induced neuronal damage in the rat hippocampus, J Appl Toxicol 1, 145-48.

Rea, T M, Nash, J F, Zabik, J E, Born, G S and Kessler, W V (1984): Effects of toluene inhalation on brain biogenic amines in the rat, Toxicology 31, 143-50.

Sapolsky, R M (1985): Glucocorticoid toxicity in the hippocampus: temporal aspects of neuronal vulnerability, Brain Res 359, 300-5.

Scatton, B, Simon, H, Le Moal, M and Bischoff, S (1980): Origin of dopaminergic innervation of the rat hippocampal formation, Neurosci Letts 18, 125-31.

Schaffer, K (1892): Beitrag zur histologie der ammonshornformation, Arch Mikroskop Anat 39, 611-32.

Segal, M and Bloom, F E (1974): The action of norepinephrine in the rat hippocampus, I, Iontophoretic studies, Brain Res 72, 79-97.

Segal, M and Bloom, F E (1976): The action of norepinephrine in the rat hippocampus, III, Hippocampal cellular responses to locus coeruleus stimulation in the awake rat, Brain Res 107, 499-511.

Selwyn, M J (1976): Triorganotin compounds as ionophores and inhibitors of ion translocating ATPases. In: Organotin Compounds: New Chemistry and Applications, Zuckerman, J J ed, pp 204-26, Advances Amer Chem Soc Ser no 157 Amer Chem Soc Publ, Washington DC.

Selwyn, M J, Dawson, A P, Stockdale, M and Gains, N (1970): Chloride-hydroxide exchange across mitochondrial, erythrocyte and artificial lipid membranes mediated by trialkyl- and triphenyltin compounds, Eur J Biochem 14, 120-6.

Seppäläinen, A M, Lindström, K and Martelin, T (1980): Neurophysiological and psychological picture of solvent poisoning, Am J Ind Med 1, 31-42.

Siesjø, B K (1981): Cell damage in the brain: A speculative synthe , J Cereb Blood Flow Metabol 1, 155-85.

Stefanis, C (1964): Hippocampal neurons: their responsiveness to microelectrophoretically administered endogenous amines, Pharmacologist 6, 171.

Stefanis, C (1968): Discussion of serotonin effects on central neurons, Advanc Pharmacol 6A, 414-18.

Storm-Mathisen, J (1972): Glutamate decarboxylase in the rat hippocampal region after lesions of the afferent fibre systems, Evidence that the enzyme is localized in intrinsic neurones, Brain Res 40, 215-35.

Storm-Mathisen, J (1977): Glutamic acid and excitatory nerve endings: reduction of glutamic acid uptake after axotomy, Brain Res 120, 379-86.

Storm-Mathisen, J (1978): Localization of putative transmitters in the hippocampal formation. In: Ciba Foundation Symposium, Vol 58 (New series): Functions of the Septo-Hippocampal System, pp 49-79, Elsevier/North-Holland, Amsterdam.

Storm-Mathisen, J (1981): Autoradiographic and microchemical localization of high affinity glutamate uptake. In: Glutamate: Transmitter in the Central Nervous System, Roberts, P J, Storm-Mathisen, J and Johnston, G A R, eds, pp 89-115, John Wiley, Chichester.

Storm-Mathisen, J and Fonnum, F (1971): Quantitative histochemistry of glutamate decarboxylase in the rat hippocampal region, J Neurochem 18, 1105-11.

Storm-Mathisen, J and Iversen, L L (1979): Uptake of [^{3}H] glutamic acid in excitatory nerve endings: light and electronmicroscopic observations in the hippocampal formation of the rat, Neurosci 4, 1237-53.

Stumpf, C (1965): Drug action on the electric activity of the hippocampus, Int Rev Neurobiol 8, 77-128.

Swanson, L W (1978): The anatomical organization of septo-hippocampal projections. In: Ciba Foundation Symposium, Vol 58 (New series), Functions of the Septo-Hippocampal System, pp 25-43, Elsevier/North-Holland, Amsterdam.

Swanson, L W (1982): The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat, Brain Res Bull 9, 321-53.

Swanson, L W (1983): The hippocampus and the concept of the limbic system. In: Neurobiology of the Hippocampus, Seifert W, ed, pp 3-19, Academic Press, London.

Swanson, L W and Cowan, W M (1977): An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat, J Comp Neurol 172, 49-84.

Swanson, L W and Cowan, W M (1979): The connections of the septal region in the rat, J Comp Neurol 186, 621-56.

Takeuchi, Y and Hisanaga, N (1977): The neurotoxicity of toluene: EEG changes in rats exposed to various concentrations, Br J Ind Med 34, 314-24.

Valdes, J J, Mactutus, C F, Santos-Anderson, R M, Dawson, R and Annau, Z (1983): Selective neurochemical and histological lesions in rat hippocampus following chronic trimethyltin exposure, Neurobehav Toxicol Teratol 5, 357-61.

Vanderwolf, C H (1969): Hippocampal electrical activity and voluntary movement in the rat, Electroenceph Clin Neurophysiol 26, 407-18.

Vertes, R P (1982): Brain stem generation of the hippocampal EEG, Prog Neurobiol 19, 159-86.

Wainer, B H, Levey, A I, Rye, D B, Mesulam, M-M and Mufson, E J (1985): Cholinergic and non-cholinergic septohippocampal pathways, Neurosci Letts 54, 45-52.

Walaas, I (1983): The hippocampus. In: Chemical Neuroanatomy, Emson, P C, ed, pp 337-58, Raven Press, New York.

Wieloch, T (1985): Neurochemical correlates to selective neuronal vulnerability, Prog Brain Res 63, 69-85.

Wieth, J O and Tosteson, M T (1979): Organotin-mediated exchange diffusion of anions in human red cells, J Gen Physiol 73, 765-88.

Yamawaki, S, Segawa, T and Sarai, K (1982): Effects of acute and chronic toluene inhalation on behavior and [^{3}H]-serotonin binding in rat, Life Sciences 30, 1997-2002.

Zimmer, L, Woolley, D and Chang, L (1985): Does phenobarbital protect against trimethyltin-induced neuropathology of limbic structures? Life Sciences 36, 851-58.

PAPER I - IV

NeuroToxicology 6 (3):145-158 (1985)

Changes in Neurobiological Parameters in the Hippocampus After Exposure to Trimethyltin

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ABSTRACT: *The effects of trimethyltin (TMT) on neurotransmitters, morphological changes and physiological activity of the hippocampus were studied. A single injection of TMT (8 mg/kg) decreased the high affinity uptake of glutamate (HA-Glu), which is a marker for glutamergic nerve terminals, after 7 days. The maximal reduction of HA-Glu was 42% and was obtained on postinjection day 21. Glutamate decarboxylase (GAD) and choline acetyltransferase (ChAT), markers for GABAergic and cholinergic structures, were not affected. The electrical activity of the hippocampus recorded through chronically implanted electrodes was altered by day three postinjection. The amplitude of the hippocampal electrographic record gradually decreased and the EEG ceased to be correlated with the rats' behavioral state. Fink-Heimer staining showed degenerating neurons within the subiculum, CA1, ventral CA3 and CA4 hippocampal subfields.*

TMT (3 mg/kg) injected once a week for three weeks decreased the HA-Glu significantly 21 days after the first injection. The HA-Glu was reduced by a maximum of 68%. The activity of ChAT was slightly increased only at day 35 postinjection while the GAD activity was not significantly reduced over a 21 day period beginning on day 14. Fink-Heimer staining showed degeneration of nerve cells within the CA1, ventral CA3 and CA4 hippocampal subfields.

Both injection schedules produced degenerating neurons in the entorhinal cortex. The neurons of the dorsal CA3 region and the granule cells of the dentate gyrus were not lesioned by either TMT injection. The relationship between the behavioral, physiological and neurochemical changes induced by TMT will be discussed.

Key words: Trimethyltin, Hippocampal EEG, High Affinity Glutamate Uptake, Neurotransmitters

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INTRODUCTION

Trimethyltin (TMT), an organotin compound, is a byproduct in the manufacture of dimethyltin chloride, a stabilizing agent for certain plastics. Exposure to TMT causes neuropathological changes in the limbic system of the brain (Bouldin *et al.*, 1981; Brown *et al.*, 1979). Several studies have demonstrated that the hippocampal pyramidal cells which are limbic system neurons, degenerate after exposure to TMT (Bouldin *et al.*, 1981; Brown *et al.*, 1979; Dyer *et al.*, 1982a; Mushak *et al.*, 1982). Degeneration of these neurons would be expected to produce changes in the neurochemical markers utilized by the affected pathways. For example, the pyramidal cells of the ventral CA3 and CA4 hippocampal subfields appear to be particularly sensitive to TMT's cytotoxic effects (Bouldin *et al.*, 1981; Brown *et al.*, 1979). These neurons project axons which synapse within the stratum radiatum of the hippocampal CA1 subfield (Hjort-Simonsen, 1973). This projection (the Schaffer collaterals) is believed to release glutamate as a neurotransmitter (Nadler *et al.*, 1976; Malthe-Sorenssen *et al.*, 1979). The high affinity uptake of glutamate (Fonnum and Walaas, 1979), which is a marker for glutamergic neurons (Fonnum *et al.*, 1981), was examined in the TMT lesioned animals. The hippocampus also contains inhibitory interneurons, the basket cells, which are believed to release gamma-aminobutyric acid (GABA) as a neurotransmitter (Storm-Mathisen, 1977). In addition the hippocampus contains cholinergic terminals derived from the medial septum and the nucleus of the diagonal bank (Fonnum *et al.*, 1970; Storm-Mathisen, 1977). Electrophysiological evidence suggests that TMT will decrease inhibition within the hippocampus (Dyer *et al.*, 1982b). It is not clear whether this reduction is due to a loss of the basket cells or their functional inhibition. Such an effect could also be mediated via the cholinergic input on the basket cells. We, therefore, utilized neurochemical assays to examine the effects of TMT on the CA3-CA4 Schaffer collateral pathway and the hippocampal inhibitory interneurons.

The hippocampal electrical activity is a well described characteristic of the hippocampus (Vanderwolf, 1969; Winson, 1974). The destruction of hippocampal pyramidal cells should alter the physiological activity of the hippocampus. In fact TMT first increases, then decrease the amplitude of hippocampal evoked potentials (Zimmer *et al.*, 1982). Hence, we recorded hippocampal electrographic activity to establish the correlation between degeneration of the pyramidal cells and physiological alterations of the hippocampus.

MATERIALS AND METHODS

Experimental Animals

Male Wistar rats were used in all experiments. The animals were housed in group cages containing 4-7 rats. Rats implanted with electrodes were placed in individual cages. The animal room was maintained on a 12 hour light-dark cycle. The rats were given food and water *ad libitum*.

Drug Injection

Trimethyltin chloride was injected on two dosing schedules. The "single" injection schedule consisted of a solitary injection of TMT chloride (8 mg/ kg, ip) in corn oil on experimental day 0. The "multiple" injection schedule involved three injections of TMT chloride (3 mg kg, ip) in corn oil at one week intervals. The injections were made on days 0, 7, 14. The injection volume in all cases, was 1 ml kg. Trimethyltin chloride was purchased from Aldrich-Europe (Belgium).

Neurochemical Assays

For uptake and enzyme activity studies the rats were decapitated, the brains removed and placed on ice. The hippocampi were dissected out and rinsed in cold physiological saline. Slices (800 μm) were cut transverse to the long axis of the hippocampus with a Sorvall tissue chopper (Model TC-2). The CA1 subfield and subiculum including stratum oriens, pyramidale, radiatum and moleculare (Fig. 1) were dissected free and homogenized in cold 0.32 M sucrose (0.5 ml) with a glass-Teflon homogenizer. Samples for the HA glutamate uptake were analyzed immediately. The homogenates were kept frozen (-20°C) and enzyme analysis performed within two days after activation of the enzyme.

The HA uptake of glutamate was chosen as a marker for glutamergic nerve terminals (Fonnum *et al.*, 1981). The determination of the HA uptake of glutamate has been described in detail elsewhere (Fonnum *et al.*, 1977). Briefly 2 μl of homogenate was added to 0.5 μl Tris Krebs medium, incubated with L-3H-glutamate for 3 min at 25°C and the uptake terminated by Millipore filtration. The amount of labelled glutamate accumulated in the tissue was measured by liquid scintillation counting.

Choline acetyltransferase (ChAT; EC 2.3.1.6) and glutamate decarboxylase (GAD; EC 4.1.1.15) were chosen as neurochemical markers for cholinergic and GABAergic nerve terminals, respectively. Previously described methods were used for determination of GAD (Fonnum *et al.*, 1970) and ChAT (Fonnum, 1975). Protein content was measured by the method of Lowry (Lowry *et al.*, 1951).

Neurophysiology

Surgical preparation of the rats (275-325 g) was conducted under diazepam (Valium, Roche) and fentanyl, fluanisone (Hypnorm, Mekos) anesthesia. The rat was placed in a stereotaxic frame, an incision made in the skin overlying the skull

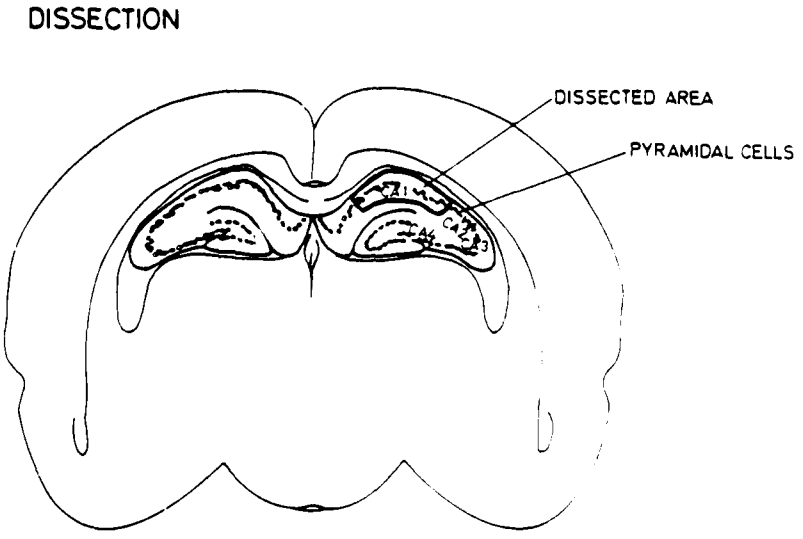

FIG. 1. The dissection for uptake and enzyme activity studies was performed on a 800 μm slice cut out at the level A 3400 μm (Konig and Klippel, 1963). The figure shows the hippocampus omitting other structures in the plane. The dissected area, which is marked in the figure consist of the CA1 subfield, including stratum oriens, pyramidale, radiatum and moleculare, and the subiculum

and the connective tissue scraped away. Stainless steel screws were set in the skull to serve as anchors. A bipolar electrode of twisted stainless steel wire (200 μm) insulated except for the tip was implanted in each hippocampus (coordinates AP + 3.2; L + -2.5; P -2.1 bregma dura, Konig and Klippel, 1963). The electrodes were covered with dental acrylic which secured the electrodes to the stainless steel screws. The rats were allowed 10 days to recover before experimentation.

The hippocampal electrical activity from awake freely moving rats was recorded through an EEG preamplifier (Grass model 7PIA) and recorded on a Grass model 7 polygraph. The signal was amplified and filtered (highpass 1 Hz, lowpass 35 Hz). Forty seconds of electrical activity were recorded every ten minutes during each hour long recording session. The single injection schedule rats were recorded on days 0, 3, 7, 14 and 21. The multiple injection rats were injected on days 0, 7, 14 and recorded prior to the injection of TMT and they were recorded on days 21 and 28. The rat's behavior was simultaneously noted on the paper record.

Histology

The rats were killed by decapitation and the brain removed. The brain was frozen using liquid carbon dioxide and sectioned (20 μm) using a refrigerated cryostat. The slices were allowed to air dry for two hours then fixed overnight using a $NaPO_4$ buffered 10% formalin solution. The slices were then stained by a modification of the Fink-Heimer method (Hjort-Simonsen, 1976). Adjacent sections were stained with Cresyl-Violet.

Statistics

The neurochemical data were initially analyzed for differences of means by an analysis of variance (ANOVA). Groups of data which contained unequal means were further analyzed by the Neuman-Keuls multiple range test. A probability of $p<0.05$ was accepted as significant in all cases (Zar, 1974).

RESULTS

Neurochemistry

Single injection schedule. The single injection of TMT significantly altered the HA uptake of glutamate within the hippocampal CA1 subfield (Table 1). The HA-uptake was significantly reduced on day 7 and continued to decrease for the next 14 days. The choline acetyltransferase and glutamic acid decarboxylase activities were not affected during this period.

Multiple injection schedule. The "multiple" injection schedule of TMT produced a progressive reduction in the high affinity uptake of glutamate (Table 2). However, this decrease did not appear until day 28. The GAD activity was not affected during the experimental period. ChAT activity was significantly increased only on day 35.

TABLE 1. The Effects of a Single Trimethyltin Chloride (8 mg/kg) Injection on Hippocampal Neurotransmitter Markers

Days After Injection	HA-Glu	GAD	ChAT
3	107±4(12)	114±5(7)	95±8(7)
7	77±5(13)*	94±6(13)	110±4(11)
14	68±5(13)*	91±5(13)	108±4(13)
21	58±5(13)**	115±6(13)	105±5(13)

All values are presented as percent of non-injected controls ±S.E.M.

*Denotes values which are significantly different from 3 day values.

**Indicates values significantly different from 3 and 7 day values (Neuman-Keuls multiple range test $p<0.05$)

The parenthesis ( ) indicate the number of animals tested

Abbreviations: HA-Glu—high affinity glutamate uptake GAD—glutamate decarboxylase ChAT—choline acetyltransferase

TABLE 2. Hippocampal Neurochemical Markers Following 3 weekly Injections (3 mg/kg) of Trimethyltin Chloride

Days After First Injection	HA-Glu	GAD	ChAT
22	96±6(9)	102±1(8)	86±10(9)
28	54±9(8)*	94±4(8)	99± 7(8)
35	32±3(8)**	104±8(8)	131±15(8)**

The results are expressed as percent of non-injected controls ±S E.M

*Denotes values which are significantly different from 1 week values.

**Denotes values significantly different from 1 and 2 week values (Neuman-Keuls multiple range test, $p<0.05$)

The parenthesis ( ) indicate the number of animals in each sample

Abbreviations: HA-Glu—high affinity glutamate uptake, GAD—glutamate decarboxylase, ChAT—choline acetyltransferase

Neurophysiology

Preinjection. The hippocampal electrical activity of awake rats consists of two distinguishable wave patterns (Fig. 2). The electrographic record of a motionless rat consists of low frequency, asynchronous waves and includes variably occurring large amplitude waves (Vanderwolf, 1969). This EEG pattern is referred to as irregular activity (IA). Rhythmic slow activity (RSA) accompanies voluntary movement and is comprised of regular synchronous electrical waves (Vanderwolf, 1969). The RSA of a walking rat has a frequency of 7-8 Hz.

Single injection schedule. The "single" injection of TMT produced progressive changes in the hippocampal electrical

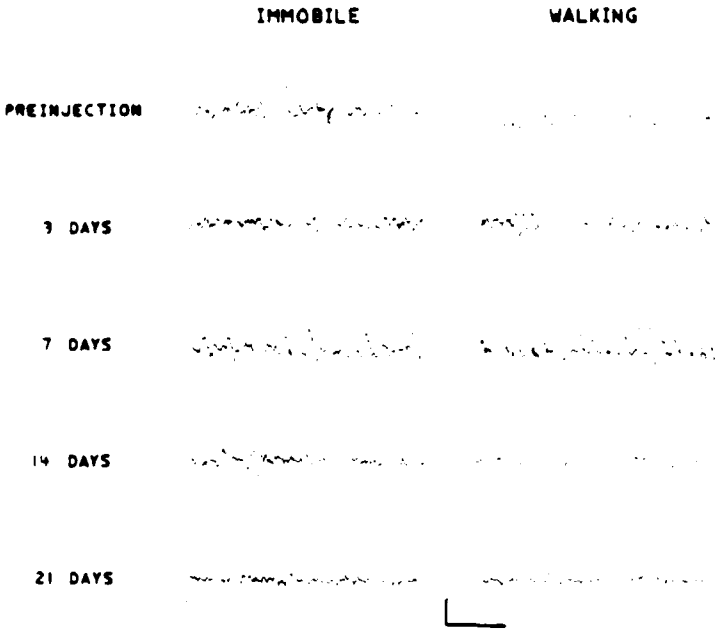

FIG. 2. The hippocampal electrical activity recorded before and after a single injection of TMT. The records are from a single rat recorded during two different behavioral states. Immobile describes a motionless but awake, alert rat. Walking refers to active movement within the recording chamber. The number of days indicate the survival time following a single injection of TMT (8 mg/kg). Note the gradual decrease in EEG amplitude with increasing survival time and also the small spikes present during immobility on day 21. The horizontal bar and vertical bar represent 1 sec and 500 uV, respectively.

activity of the treated rats (Fig. 2). Three days after the TMT injection both the IA and the RSA amplitude were decreased and the frequency reduced. By day 7 the RSA frequency during walking had decreased from 7-8 Hz to 5-6 Hz. At the same time the electrographic record during alert motionless behavior showed an increased number of slow waves. The RSA amplitude on day 14 was decreased 50% to 60% compared to preinjection controls although regular waves were present. The large slow waves characteristic of IA were almost completely absent having been replaced by low amplitude fast waves. On day 21, the electrical activity recorded during voluntary movement consisted of very low amplitude waves which did not change when the rat became motionless. However, the EEG of a still rat contained spikes which occurred every 5 to 20 seconds.

Multiple injection schedule. The multiple injection schedule of TMT produced gradual changes in the electrical activity of the treated rats (Fig. 3). The electrographic records on day 7 contained RSA and IA which appeared normal. On day 14 the RSA amplitude appeared to be slightly increased and the frequency during walking decreased to 5-6 Hz. The irregular activity during motionless but alert behavior was also increased in amplitude and contained more large slow waves. The EEG characteristics changed dramatically between day 14 and day 21. The hippocampal electrical activity consisted of low amplitude slow waves which did not change as the rats behavior

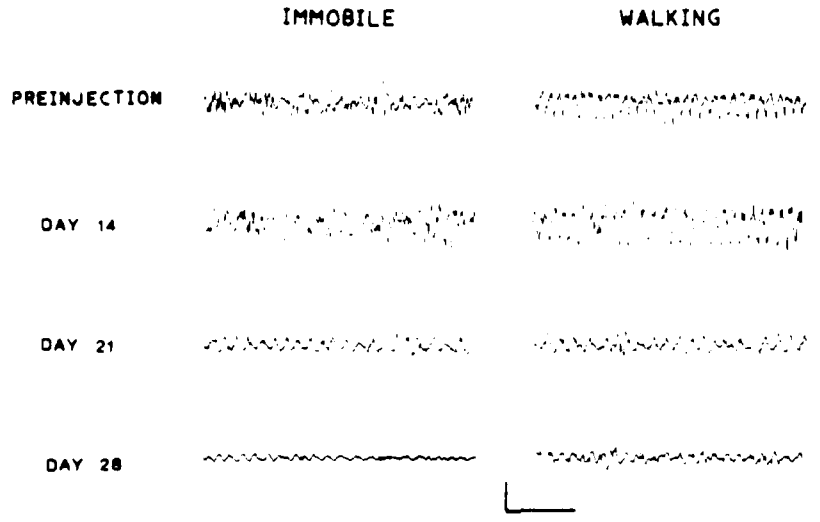

FIG. 3. The hippocampal electrical activity before and after a multiple TMT injection schedule. The records are from a single rat recorded during two different behavior states. The animals received 3 TMT injections (3 mg/kg) spaced one week apart. On days of injections the recordings were done prior to injection. Immobile describes a motionless but awake and alert rat. Walking refers to active movement within the recording chamber. The times indicate the duration since the first TMT injection. Beginning with day 21 the EEG does not change as the rat's behavior changes. The horizontal bar and vertical bar represent 1 sec and 500 uV.

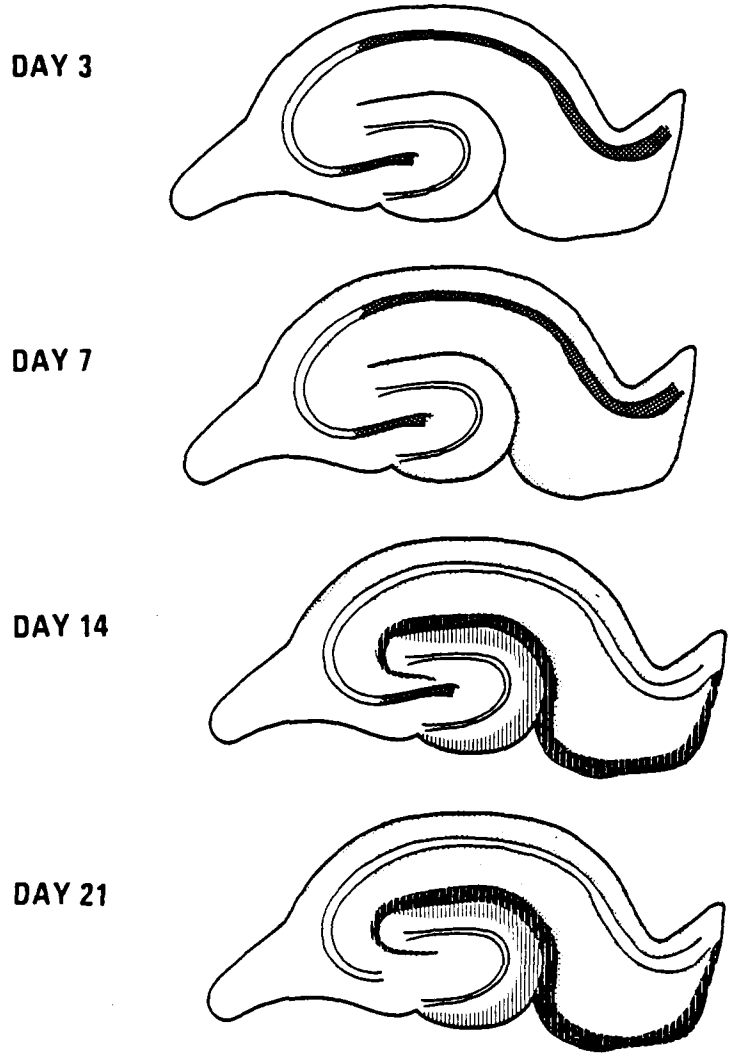

FIG. 4. Patterns of staining for degenerating neurons following a single TMT injection. Cross-hatching refers to degenerating pyramidal cells within the CA1, subiculum, ventral CA3, and CA4. The fine stippling indicates those *dendritic areas showing evidence of heavy degeneration. The white vertical* lines with a black background are degenerating axons within the perforant pathway. The staining in this area is lighter than that in the stippled area. The dark vertical lines with a white background is a lighter staining region in the outer 2/3 of the molecular layer of the dentate gyrus. These are probably degenerating terminals of the perforant pathway.

changed. The waves had a frequency of 4-6 Hz and an amplitude approximately half of the control RSA amplitude. The EEG of walking and immobile rats on day 28 was comprised of only low amplitude (<150 uV) fast waves which showed no correlation to the animals behavior.

Histology

Single injection schedule. Three days

after the injection of a single large dose of TMT Fink-Heimer staining provided evidence of degenerating axons within CA1, ventral CA3 and CA4 hippocampal subfields. The subiculum showed only occasionally damaged structures. The resistance of the dorsal CA3 pyramidal cells to TMT's neurotoxic effects is similar to that reported previously (Bouldin *et al.*, 1981; Brown *et al.*, 1979; Valdes *et al.*, 1983). A slight degeneration was present in the stratum radiatum and the stratum oriens of CA1. The entorhinal cortex contained neuronal cell bodies showing signs of degeneration.

Seven days postinjection the ventral CA3 and the CA4 subfields still contained the largest amount of degeneration (Fig. 4). There were signs of increased degeneration within the CA1 subfields. The stratum oriens, stratum radiatum and the stratum lacunosum-moleculare showed signs of degeneration. Additionally there was evidence of damaged nerve tissue in the outer portion of the molecular layer of the dentate gyrus. This may represent degenerating perforant pathway terminals originating from entorhinal cortex neurons (Hjort-Simonsen and Jeune, 1972). The cresyl-violet stained sections of day 21 showed the absence of neurons in the ventral CA3 and CA4 subfields.

Multiple injection schedule. The multiple TMT injection schedule produced a pattern of degenerating neurons different from the single injection schedule (Fig. 6, 7). On day 21, the ventral CA3 and the CA4 subfield contained evidence of degenerating neurons (Fig. 5). A week later, evidence of damaged neurons was found in the CA1 in addition to the ventral CA3 and CA4 subfields. The neurons within the subiculum and the dorsal CA3 appeared to be insensitive to TMT's neurotoxic effects. The perforant pathway and its terminal field, the outer 2/3 of the molecular layer of the dentate gyrus and molecular layer of hippocampus showed signs of damaged nervous tissue. On day 35 only the dendritic regions showed darkly staining areas. The cresyl violet stained sections showed an absence of nerve cell bodies in the ventral CA3 or the CA4 pyramidal cell areas and also a loss of CA1 neurons (Fig. 7).

DISCUSSION

Trimethyltin produces physiological, morphological and biochemical changes within the hippocampus. Following a single TMT injection the histological and physiological changes occurred within 3 days while the biochemical alterations occurred after 7 days. With the multiple injection schedule the EEG was changed after 14 days while the histological and biochemical parameters were changed after 28 days.

The CA4 pyramidal cells and the ventral CA3 pyramidal cells are the most sensitive to the toxicant actions of TMT. These pyramidal cells have almost completely disappeared by 21 days after a single TMT injection or by 35 days after beginning the multiple injection schedule. The biochemical or morphological characteristics which make this subfield so sensitive to TMT exposure are unknown. The CA1 pyramidal cells are less sensitive to TMT induced degeneration. Although neurons within this subfield degenerate they do not completely disappear (Fig. 6; Dyer *et al.*, 1982a). The pattern of TMT induced degeneration of the CA1 and CA4 pyramidal cells is similar to that of previous investigations (Brown *et al.*, 1979, Bouldin *et al.*, 1981, Mushak *et al.*, 1982, Valdes *et al.*, 1983). The sparing of the dorsal CA3 neurons observed in the present study has been observed by these research groups although a progressive degeneration of the pyramidal cells beginning in the CA4 region and extending as far as the CA2 (Dyer *et al.*, 1982) has also been reported. A combination of differences between dosing regimens, injec-

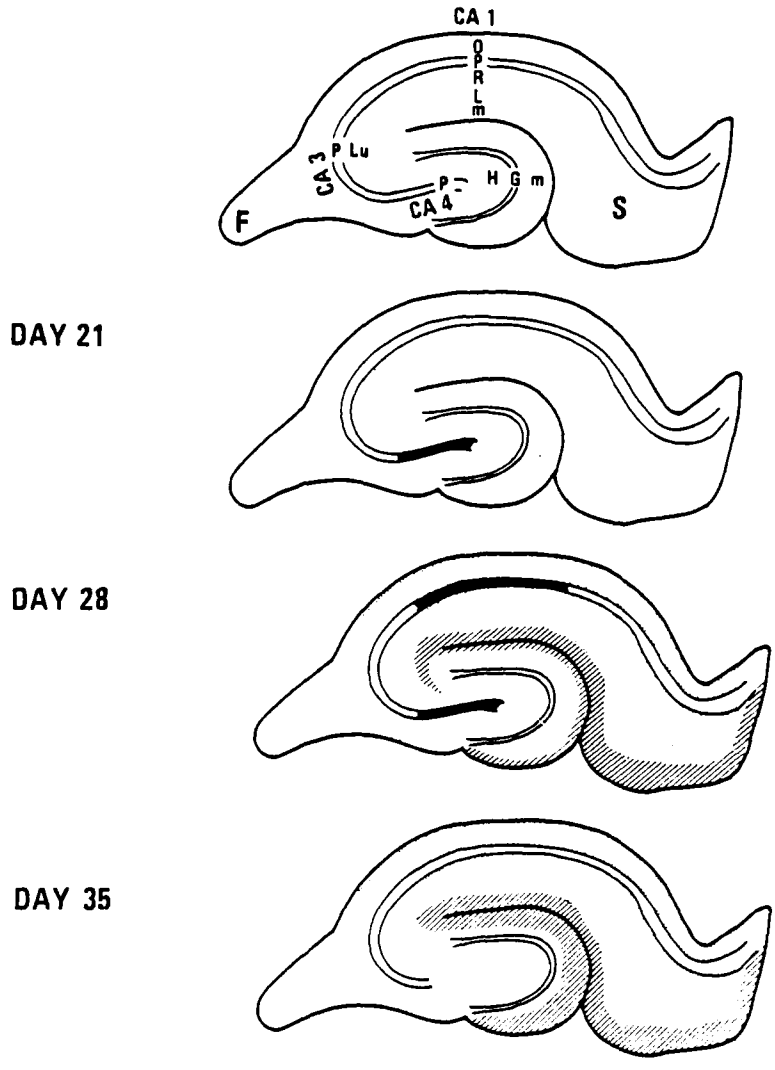

FIG. 5. Schematic drawing of the hippocampus showing the degenerating pattern in major subdivisions after multiple TMT injections. The black regions indicate areas containing degenerating pyramidal cells. The stippling refers to areas heavily stained for the presence of degenerating fibers. The dark slanted lines indicate lighter staining degenerating areas. Abbreviations: S—subiculum, F—fimbria, P—pyramidal cell body layer, O—stratum oriens, R—stratum radiatum, L,m—stratum lacunosum-moleculare, Lu—stratum lucidum, H—hilus of fasciae dentatae, G—dentate granule cells, m—molecular layer of the dentate gyrus, CA1, CA3 and CA4—subfields of the hippocampal pyramidal cells

tion vehicle and time of sacrifice probably produce different absorption rates and a final brain TMT concentration differentially affecting the dorsal CA3 region.

The degeneration of pyramidal cells will reduce the markers for the neurotransmitters utilized by these neurons. The CA3 and CA4 pyramidal cells pro-

FIG. 6. Cresyl-violet stained sections before (A and C) and 35 days after (B and D) the multiple TMT injections schedule (3 x 3 mg/kg). Note the decreased width in the CA1 pyramidal cell subfield and the absence of pyramidal cells in the ventral CA3 and CA4 regions. C and D shows the CA1 area. D shows a reduction in number of pyramidal cells and an increase in glial cells in the dendritic region.

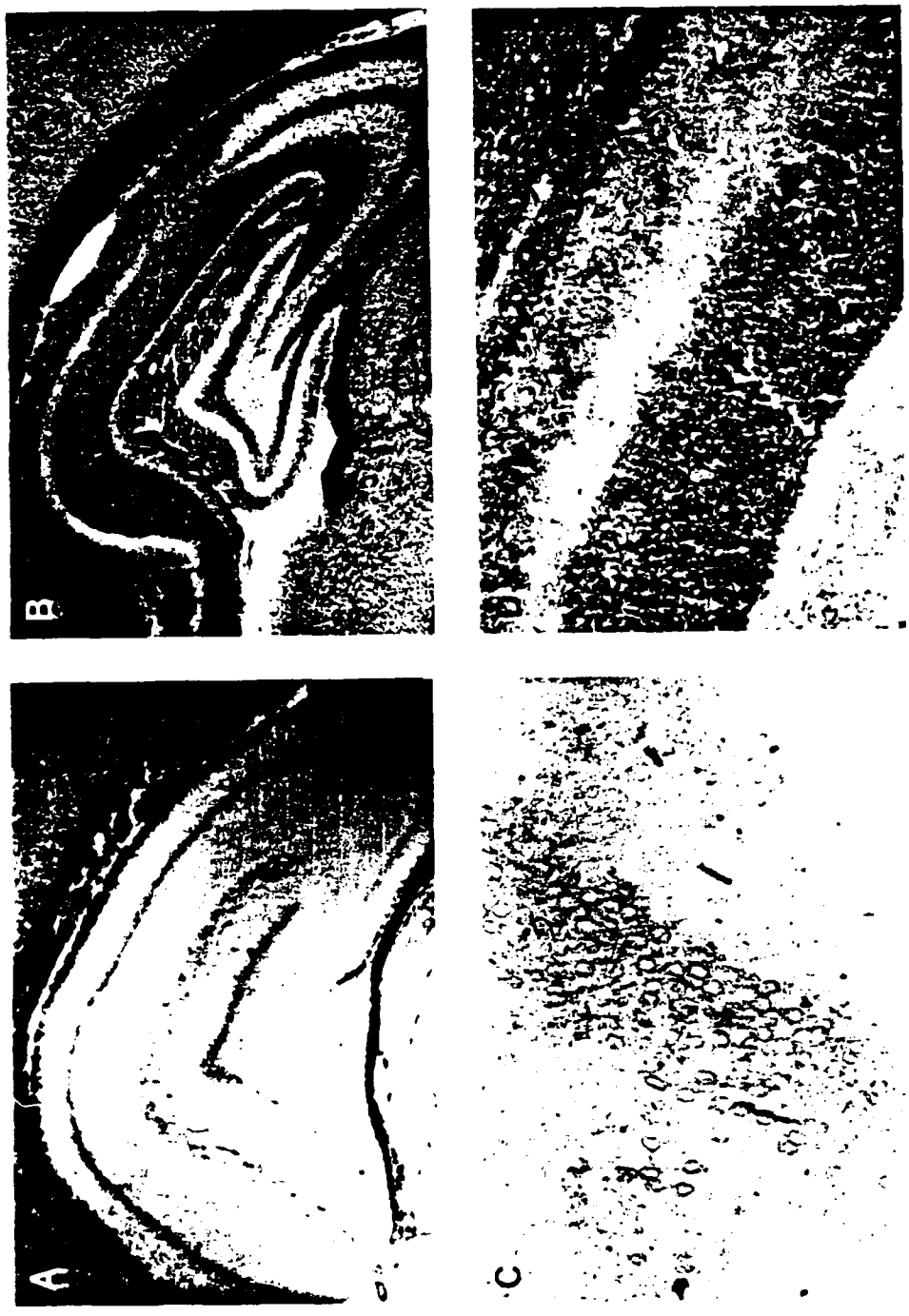

FIG. 7. Fink-Heimer stained sections before (A and C) and 35 days after (B and D) a multiple TMT injection schedule (3 x 3 mg/kg). The dark staining in the dendritic sections indicate massive degeneration. The cell body layer of CA1, CA3 and CA4 subfields seems completely absent. C and D shows the CA1 area

ject axons, the Schaffer collaterals, which synapse on dendrites of the CA1 neurons (Hjort-Simonsen, 1973). The terminals of the CA3 and CA4 neurons are believed to release glutamate as a neurotransmitter (Nadler *et al.*, 1976; Malthe-Sorenssen *et al.*, 1979). The first signs of degenerating cell bodies appear in the CA3 and CA4 region 3 days after a single dose of TMT (8 mg/kg, ip). At this time the high affinity glutamate uptake, a marker for glutamergic terminals, remains at control levels. The HA Glu is reduced 25% seven days postinjection (Table 1). Simultaneously there is evidence of degeneration within the stratum radiatum, the terminal zone for the Schaffer collaterals. Although it would be nice to say we can identify degenerating terminals the staining within the stratum radiatum probably includes degenerating dendrites of the CA1 neurons. However, the data clearly demonstrates that the CA3 and CA4 cell bodies show the first degeneration after TMT exposure followed by degeneration in the terminal fields. The data reported here supports the conclusion of Valdes *et al.*, (1983) that the decreased high affinity uptake of glutamate is secondary to the loss of pyramidal cell neurons.

The activity of glutamic acid decarboxylase, a specific marker for GABAergic terminals is not altered by TMT exposure. These observations suggest that the basket cells, inhibitory interneurons within the CA1 subfield are not lesioned by TMT. Several authors have suggested based on physiological and biochemical data that TMT alters the activity of the inhibitory interneurons. For example, TMT exposure reduces the concentration of GABA within the hippocampus (Mailman *et al.*, 1983) suggesting that TMT will affect the GABAergic systems. The decreased GABA concentration may be secondary to a reduction in the high affinity uptake of 3H-GABA which appears to occur in a nonspecific manner (Doctor *et al.*, 1982). The decreased GABA levels may be the mechanism which reduces the recurrent inhibition of the CA3 region following exposure to TMT (Dyer *et al.*, 1982b).

Trimethyltin in addition to killing pyramidal cells will alter the physiological activity of the hippocampus (Allen and Fonnum, 1984). TMT reduces the amplitude of dentate granule cell evoked potentials generated by stimulation of the pyriform cortex (Zimmer *et al.*, 1982). The activation of the granule cells by pyriform cortex stimulation is via a pathway which projects from the pyriform cortex and synapses upon neurons of the entorhinal cortex (Hjort-Simonsen, 1973). The entorhinal neurons project axons via the perforant pathway synapsing on the granule cells of the dentate gyrus. Zimmer and co-workers (Zimmer *et al.*, 1982) postulated that the transmission failure occurred within the entorhinal cortex. The histological data presented here supports the idea that the reduction in granule cell evoked potentials is due to a degeneration of entorhinal cortex neurons and axons of the perforant pathway. The degeneration within the outer 2/3 of the molecular layer represents terminal degeneration of the perforant pathways since this is the area where they are known to synapse (Hjort-Simonsen, 1973). The evoked potentials reach a very low amplitude between 8 to 20 days post injection. Using a similar dose (8.0 mg/kg vs. 7.5 mg/kg by Zimmer *et al.*, 1982) we observed degenerating axons beginning on day 14. However, neurons of the fascia dentata are not immune to TMTs effects. Bouldin *et al.*, (1981) reported that short term high doses (5 mg/kg/day, 3 days) produced damage which is limited to granule cell neurons in the fascia dentate while in low dose long term (1 mg/kg on alternate days, 14 injections) animals the neuronal loss was much more marked in the pyramidal neurons of the hippocampus. Brown *et al.* (1979) have also reported

damaged cells of the fascia dentata after a single high dose injection. Damage to the granule cells contributing to the degeneration seen in the molecular layer could not be ruled out.

The hippocampal electrical activity recorded in these experiments is an extracellular correlate of the activity of intracellular potentials. For example, the rhythmic slow activity generator appears to be within the cell body layer (Bland *et al.*, 1976; Green *et al.*, 1979). Fujita and Sato (1964) demonstrated the presence of an intracellular RSA which was composed of excitatory postsynaptic potentials. The generation of the electrical waves is thought to be a combination of excitation of the pyramidal cells and an inhibition by the basket cells (Kandel *et al.*, 1961; Anderson and Eccles, 1962). These observations show that an intact hippocampal neuronal circuitry is necessary for the generation of normal hippocampal electrical activity. The loss of pyramidal cells after TMT exposure would be expected to alter the physiological activity of the hippocampus. TMT exposure alters the electrical activity of the hippocampus within 3 days (Fig. 2; Ray, 1981). These alterations occur at a time when the first signs of degeneration appear in the hippocampus (Fig. 4). Ray (1981) recorded an increased RSA amplitude from the granule cells on days 2-4 following a single 10 mg/kg oral dose of TMT. These observations contrast with the decreased amplitude of the present studies (Fig. 2). These opposite findings suggest differential effects of TMT on the CA1 pyramidal cells and the granule cell neurons. The dysfunction of the hippocampus electrical activity may provide a clue to the deficits in behavioral task performance following TMT treatment (Miller *et al.*, 1982; Swartzwelder *et al.*, 1982; Walsh *et al.*, 1982).

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REFERENCES

Allen CN, Fonnum F. Trimethyltin inhibits the activity of hippocampal neurons recorded *in vitro*. *Neurotoxicology* 1984; 5(4):23-30

Anderson P, Eccles J. Inhibitory phasing of neuronal discharge. *Nature* 1962; 96:645-647

Bland BH, Whishaw IQ. Generators and topography of hippocampal theta (RSA) in the anaesthetized and freely moving rat. *Brain Res* 1976; 118:259-280

Bouldin TW, Goines ND, Bagnell CR, Krigman MR. Pathogenesis of trimethyltin neuronal toxicity: Ultrastructural and cytochemical observations. *Am J Path* 1981; 104:237-249

Brown AW, Aldridge WN, Street BW, Verschoyle RD. The behavioral and neuropathological sequelae of intoxication by trimethyltin compounds in the rat. *Am J Path* 1979; 97:59-82

Doctor SV, Costa LG, Kendall DA, Murphy SD. Trimethyltin inhibits uptake of neurotransmitter in mouse forebrain synaptosomes. *Toxicology* 1982; 25:213-221

Dyer RS, Deshields TL, Wonderlin WF. Trimethyltin-induced changes in gross morphology of the hippocampus. *Neurobehav Toxicol Teratol* 1982a; 4:141-147

Dyer RS, Wonderlin WF, Walsh TJ, Boyles WK. Trimethyltin reduces basket cell inhibition in the dentate gyrus. *Neurosci Abs* 1982b; 8:82

Fonnum F, Walaas I. The effect of intra-hippocampal kainic acid injections and surgical lesions on neurotransmitters in the hippocampus and septum. *J Neurochem* 1979; 31:1173-1181

Fonnum F, Storm-Mathisen J, Walberg F. GAD in inhibitory neurons. A study of the enzyme in Purkinje cell axons. *Brain Res* 1970; 259-275

Fonnum F. A rapid radiochemical method for determination of choline acetyltransferase. *J Neurochem* 1975; 24:407-409

Fonnum F, Walaas I, Iversen E. Localization of GABAergic, cholinergic and aminergic structures in the mesolimbic system. *J Neurochem* 1977; 29:221-230

Fonnum F, Malthe-Sorenssen D, Kvale I, Soreide A, Skrede KK, Walaas I. Glutamergic neurons. Localization and release of the transmitter. In: *Regulatory Mechanisms of Synaptic Transmission. Tapia R, Cotman CW, eds. Plenum Publishing Corp. 1981; 59-70*

Fujita Y, Sato T. Intracellular records from hippocampal pyramidal cells in rabbit during theta rhythm activity. *J Neurophysiol* 1964; 27:1011-

1025

Green K, Rawlins JNP. Hippocampal theta in rats under urethane: Generators and phase relations. *Electroencephalogr Clin Neurophysiol* 1979; 47:420-429

Hjort-Simonsen A. Fink-Heimer silver staining of degenerating axons and terminals in mounted cryostat sections of fresh and fixed brains. *Stain Technology* 1976; 5:199-204

Hjort-Simonsen A. Some intrinsic connections of the hippocampus in the rat: An experimental analysis. *J Comp Neurol* 1973; 147:145-162

Hjort-Simonsen A, Jeune B. Origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. *J Comp Neurol* 1972; 144:215-232

Kandel ER, Spencer WA, Brinley FJ. Electrophysiology of hippocampal neurons. Sequential invasion and synaptic organization. *J Neurophysiol* 1961; 24:225-242

Konig JFR, Klippel RA. The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem. The Williams and Wilkins Company, Baltimore 1963

Lowry OH, Rosebrough NJ, Farr L, Randell RJ. Protein determination with the folin phenol reagent. *J Biol Chem* 1971; 193:265-275

Mailman RB, Krigman MR, Frye GD, Hanin I. Effects of postnatal trimethyltin or triethyltin treatment on CNS catecholamines, GABA and acethylcholine systems in the rat. *J Neurochem* 1983; 40:1423-1429

Malthe-Sorenssen D, Skrede KK, Fonnum F. Calcium dependent release of D-^{3}H aspartate evoked by selective electrical stimulation of excitatory afferent fibers to the hippocampal cells *in vitro*. *Neurosci* 1979; 4:1255-1263

Miller DB, Eckerman DA, Krigman MR, Grant LD. Chronic neonatal organotin exposure alters radial-arm maze performance in adult rats. *Neurobehav Toxicol Teratol* 1982; 4:185-190

Mushak P, Krigman MR, Mailman RB. Comparative organotin toxicity in the developing rat: somatic and morphological changes and relationship to accumulation of total tin. *Neurobehav Toxicol Teratol* 1982; 4:209-215

Nadler JV, Vaca KW, White WF, Lynch GS, Cotman CW. Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents. *Nature* 1976; 260:538-540

Ray DE. Electroencephalographic and evoked response correlates of trimethyltin induced neuronal damage in the rat hippocampus. *J Appl Toxicol* 1981; 1:145-148

Swartzwelder HS, Hepler J, Holahan W, King SE, Leverenz HA, Miller PA and Myers RD. Impaired maze performance in the rat caused by trimethyltin treatment: Problem-solving deficits and preservation. *Neurobehav Toxicol Teratol* 1982; 4:169-176

Vanderwolf CH. Hippocampal electrical activity and voluntary movement in the rat. *Electroenchepal Clin and Neurophysiol* 1969; 26:407-418

Valdes JJ, MacTutus CF, Santos-Anderson RM, Danson R and Annau Z. Selective neurochemical and histological lesions in rat hippocampus following chronic trimethyltin exposure. *Neurobev Toxicol Teratol* 1983; 5:357-361

Walsh TJ, Miller DB and Dyer RS. Trimethyltin, a selective limbic system neurotoxicant, impairs radial-arm maze performance. *Neurobehav Toxicol Teratol* 1982; 4:177-183

Winson J. Patterns of hippocampal theta rhythm in the freely moving rat. *Electroencephalogr Clin Neurophysiol* 1974; 36:291-301

Zar J. *Biostatistical Analysis.* New Jersey, Prentice Hall, Inc., 1974, pp 151-154

Zimmer L, Zuheir H, Woolley D and Chang L. Limbic evoked potentials and brain levels of tin after a single dose of trimethyltin in the rat. *Neurosci Abs* 1982; 8:971

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THE EFFECT OF TRIMETHYLTIN ON THREE GLUTAMERGIC AND GABAERGIC TRANSMITTER PARAMETERS IN VITRO; HIGH AFFINITY UPTAKE, RELEASE AND RECEPTOR BINDING.

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ABSTRACT

The effects of trimethyltin (TMT) on high-affinity uptake, release and sodium-independent binding of glutamic acid and γ-aminobutyric acid (GABA) were studied in vitro in homogenates of hippocampal tissue. TMT (10 μM) increased the release of glutamic acid from synaptosomes in the resting state (5 mM K^+), whereas the release of CABA was only sligthly affected. High affinity uptake of glutamate was inhibited by TMT in the same concentration range as release. The uptake of GABA was only affected by TMT-concentrations from 500 μM to 5 mM. The sodium independent binding of both glutamate and GABA, usually assumed to be binding to receptor sites, were inhibited with 50 μM or more TMT in the incubation medium. The results indicate that TMT can interfere with several different events of the neurotransmission process in the central nervous system at concentrations which can be obtained in the brain of rats after a sublethal dose of the compound.

Running title: In Vitro effects of trimethyltin

Key words: Trimethyltin,Hippocampus, In Vitro-study, Glutamate Release.

INTRODUCTION

Many organic tin compounds are now in widespread use. In industry organotins are used as stabilizers in certain plastic polymers, as catalysts for polyurethane formation, silicone curing, esterification, epoxy curing, olefine polymerization, etc. Some organotin compounds are known to have biocidal effects and are used as fungicides, bactericides, algicides, helmintics, insecticides and chemosterilants, (Barnes and Stoner, 1959; Environmental Protection Agency, 1976).

The most toxic organotin analoges are triethyltin (TET) and trimethyltin (TMT), which are absorbed via the skin and gastrointestinal tract. The other alkyl and aryl tin compounds are poorly absorbed. TET-acetate has a LD50 value of 4 mg/kg when given orally to rats, while for TMT-acetate the value is 9.1 mg/kg. The corresponding values for higher tin analoges are for propyl, 118.3; butyl, 380.2; hexyl, 1000; octyl > 1000 (Watanabe, 1980).

Exposure to triethyltin (TET) is known to produce cerebral edema and demyelinization in the central nervous system (Barnes and Stoner, 1959; Aleu et al., 1963). Unlike the other alkyltins TMT produces neuronal necrosis rather than intramyelinic edema (Brown et al., 1979). At low doses the hippocampal formation and pyriform cortex seem to be the most vulnerable regions (Brown et al., 1979; Bouldin et al., 1981). The hippocampal pyramidal cells have been demonstrated to degenerate selectively after TMT-exposure (Brown et al., 1979; Bouldin et al., 1981; Naalsund et al., 1985; Dyer et al., 1982). We have previously measured different neurobiological parameters in the hippocampus of TMT-exposed rats and found that the glutamergic pathways are degenerating while the cholinergic and gabaergic pathways are not affected (Naalsund et al., 1985). Histological changes were detected three days after injection of a single dose (8 mg/kg i.p.) and the hippocampal EEG changed gradually from day three after the TMT-injection (Naalsund et al., 1985). A reduction in a glutamate transmitter parameter (high affinity uptake) as an indicator of degeneration of glutamergic terminals, was observed 8 days after injection.

In an in vitro recording of the electrically evoked potential of the pyramidal cells in the hippocampus TMT was shown to depress the activity of the postsynaptic CA1 neurons in a dose dependent manner at concentrations from 3.6 µg to 10 µg/ml (Allen and Fonnum,1984). In the present study the aim was to investigate the in vitro effects of TMT on the biochemical prosesses of transmission in neuronal tissue. The neuronal degeneration seen in the hippocampus after trimethyltin exposure could be a result of the disruption of neurotransmitter functions through an effect on some basic cellular functions. We present here the results of the effect of trimethyltin in vitro on the high affinity uptake, spontanous release from synaptosomes and on receptor binding of tritiated L-glutamic acid and γ-aminobutyric acid.

MATERIALS AND METHODS

CHEMICALS

Trimethyltin-chloride, 9%, (Aldrich-Europe) was dissolved to 50 mM concentration in distilled water and added in suitable volumes to the test media to give the desired concentrations. L-[2,3-^{3}H] glutamic-acid, 20 Ci/mmol, D-[2,3-^{3}H] aspartic acid, 14 Ci/mmol, γ-[2,3-^{3}H(N)] aminobutyric acid (GABA), 30 Ci/mmol, were all obtained from New England Nuclear.

TISSUE PREPARATION

Tissue homogenates were prepared from the hippocampus dissected from the brain of young adult male Wistar rats (150-250 g). The rats were killed by decapitation, the brains were removed and placed on ice. The hippocampi were dissected and homogenized in cold sucrose (0.32 M) at 800 rev/min with a glass-teflon homogenizer. These homogenates were used for uptake and release experiments.

For the experiments on synaptosomal and mitochondrial fractions, a modification of the procedure described by Gray and Whittaker (1962)

was used. Homogenates in 0.32 M sucrose were centrifugated at 1000 xg for 10 min and the pellets washed once. The combined supernatants were then centrifugated at 20 000 xg for 20 min and the P2-pellets were resuspended in 0.32 M sucrose and placed on a discontinous density gradient consisting of 0.8, 1.2 and 1.6 M sucrose. Separation was achieved by centrifugation on a swing-out head in an ultracentrifuge at 150 000 xg for 30 min. The gradients were carefully collected through a hole at the bottom of the tube. The layers between 1.6 and 1.2 M were collected as the mitochondrial fraction and the layers between 0.8 and 1.2 M as the synaptosomal fraction.

NEUROTRANSMITTER RELEASE

Release of [^{3}H] glutamic acid and [^{3}H] GABA was measured by a modification of the method described by Hammerstad et al. (1979). Briefly 2 µl homogenate containing about 2 µg protein were absorbed to a small piece of millipore filter. The filters were placed in a small tissue culture dish, four filters in each dish, and 4 ml Krebs-Hepes buffer containing 27,5 mM Hepes, 138 mM NaCl, 2,4 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM KH_2PO_4, 1,6 mM glucose were added. The synaptosome pools were labelled with [^{3}H] glutamic acid, [^{3}H] aspartic acid or [^{3}H] GABA by incubation in the Krebs-Hepes buffer containing 5 µM labeled transmitter (20-30 Ci/mmol) for 20 min at 25°C. The filters were washed four times in Krebs-Hepes buffer and then each one placed in a small perfusion chamber made of two disposable plastic tips. The chambers were continously perfused with Krebs-Hepes buffer.The liquid flow was held constant at 0.4 ml/min and perfusate was collected in fractions of 0.8 ml. The chambers were washed for 20 min with Krebs-Hepes buffer and the release studied with Krebs-Hepes buffer containing 50 mM K^+ in stead of an equivalent amount of NaCl or Krebs-Hepes buffer containing different concentrations of trimethyltin (10^{-5} - 10^{-3} M) for 4 min periods. The fractions (0.8 ml) of the perfusate were added 10 ml Insta-Gel (Packard) and the radioactivity were counted. The filters were dissolved in Filter Count (Packard) and the rest activity measured. The results were calculated as percentage fractional release. The fractional release during perfusion with wash

buffer (5 mM K^+) immidately before TMT perfusion were set to 100% and changes in fractional release were expressed in per cent of this normal spontanous release. Eight superfusion assays were run parallell by the use of an eight channel peristaltic pump. Two assays with potassium stimulation (50 mM) were run each time as positive controls of the method. The other six were subject to TMT-perfusion for four minutes pulses.

HIGH AFFINITY UPTAKE OF NEUROTRANSMITTER

High affinity uptake of $[^3H]$ glutamic acid and $[^3H]$ GABA was measured by the method described by Fonnum et al., 1980. 2 µl homogenate (containing ca 6 µg protein) were added to 0.5 ml Tris-Krebs medium containing 15 mM Tris, 140 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 10 mM glucose and pH adjusted to 7.4. The TMT was added to the Tris-Krebs buffer before addition of the tissue. The mixtures were preincubated for 15 min before incubation with 70 nM tritiated transmitter (20-30 Ci/mmol) for 3 min at 25°C. The uptake was terminated by filtration in a Titertek cell harvester with a Titertek filtermat. The filters were dissolved in 5 ml Filter Count (Packard) and the radioactivities were counted. For each animal triplicates of the high affinity uptake were performed at each TMT concentration and of TMT-free controls.

RECEPTORBINDING

We have measured the sodium-independent binding of transmitter to synaptosomal membranes. This binding is commonly referred to as the receptor binding (Foster and Fagg,1984). Binding of $[^3H]$ glutamic acid and $[^3H]$ GABA was measured by a modification of the method described by Hill et al. (1984). The hippocampi were homogenized in 20 volumes 0.32 M sucrose, centrifugated at 1000 xg for 10 min and the pellets were washed once. The combined supernatants were then centrifugated at 20 000 xg for 20 min and the pellets were resuspended to the same volume with cold, destilled water and kept on ice for one hour. After centrifugation at 8000 xg for 20 min the supernatants were gently decanted and the upper layer of the pellets were rinsed off and added

to the supernatant. These combined supernatants were recentrifugated at 48 000 xg for 20 min, and the pellets were stored frozen at -20°C prior to use.

For the binding assay, membranes were thawed and resuspended in Tris-HCl buffer (50 mM, pH 7.4) and incubated for 45 min at room temperature before centrifugation (8500x g for 10 min). The membrane suspensions were further washed three times and incubated with the Tris-buffer for 15 min at room temperature between each centrifugation. The final pellets were resuspended to give a protein concentration of about 20-80 µg per assay. Binding of [^{3}H] glutamic acid or [^{3}H] GABA (final concentrations 100 and 50 nM with a specific activity of 20 and 30 Ci/mmol respectively, or varying ligand concentrations in saturation studies) were assayed in absence or presence of 1 mM unlabeled transmitter, to determine the specific transmitter binding. Varying concentrations of trimethyltin were added to the mixture before a preincubation period of 15 min, and then after addition of the ligand the mixtures were incubated for 15 min at 30°C for glutamic acid (Baudry and Lynch, 1980) and for 10 min at 20°C for GABA (Hill et al., 1984). Binding was terminated by Titertek filtration. The filters were dissolved in Filter Count (Packard) and the radioactivities were counted. For each animal were run four assays with total binding and two assays with unspesific binding at each TMT concentration as well as of TMT-free controls.

PROTEIN MEASUREMENTS

Protein content in the homogenates were measured by the method of Lowry (Lowry et al., 1951).

SCINTILLATION COUNTING

Counting efficiency was 40% for the perfusates and 50% for filters dissolved in Filter Count.

STATISTICS

In release experiments each assay was used as its own control. For high affinity uptake and binding studies the results were compared in groups and each group compared with one common control group. The results were analyzed for differences of means by an analysis of variance (ANOVA). Groups of data which contained unequal means were further analyzed by Dunnet's test. A probability of $p<0.05$ was accepted as significant in all cases.

RESULTS

TRANSMITTER RELEASE

$[^3H]$ glutamate was released by perfusion with TMT at concentrations from 0.05 mM to 1 mM (table 1). The release was measured by exposure of homogenates in the resting state to TMT, i.e. at a potassium concentration of 5 mM. D-$[^3H]$ aspartate, commonly used as a false transmitter for glutamate (Fonnum, 1984) behaved similiar. This compound which is not metabolised, gave as expected higher releases. The release of $[^3H]$ GABA was studied in parallell and was not significantly higher than normal spontanous release in the presence of TMT in the perfusionbuffer at concentrations from 10 mM to 1 mM. The addition of different concentrations of TMT in the potassium depolarization buffer (50 mM K^+) did not give significant changes in the release of transmitters investigated (data not shown).

REPETITIVE STIMULATION

Several stimulations with high potassium concentrations (50 mM) reduced the fractional release of glutamate (figure 1). The effect of TMT induced release was similiar. A previous potassium stimulated release did not affect the amount of glutamate released by a TMT stimulation (0.1 mM), and the potassium stimulated release was not reduced by a previous TMT stimulation (figure 1).

Ca-DEPENDENCE

The release was normally performed in an assay medium containing 2 mM calcium. A replacement of the calcium chloride with magnesium sulfate (10 mM) resulted in a 45% ± 7% (N=10) decrease in release after depolarization with potassium. The effect of the calsium concentration in the perfusionbuffer on the release induced by TMT was a 24% ± 12% (N=9) decrease in release.

EFFECT ON SUBCELLULAR FRACTIONS

The mitochondria and synaptosomes were isolated in two fractions to see whether the transmitter released by TMT exposure might be derived from the mitochondria rather than from the synaptosomes. The amount of uptake and release of tritiated transmitter in the mitochondrial fraction was 12% of the amount in the synaptosomal fraction. Fractional release from the two fractions were similar both after exposure to 0.5 mM TMT and potassium stimulation. The small release from mitochondrial fraction may well be due to the presence of contaminating synaptosomes.

Na-CHANNELS

Tetrodotoxin (TTX) was used to study the importance of Na-channels (Narahashi, 1984; Catterall, 1984) in the TMT induced transmitter release. The results showed that the release by veratridine was reduced to 9% ± 2% (N=4) by TTX as expected. In contrast potassium (50 mM) stimulated and TMT (0.5 mM) stimulated release were not affected by TTX. These results suggest that the Na-channel is not involved in the release of transmitter mediated by TMT.

TISSUE CONCENTRATION

The effect of tissue concentration on the amount of $[^3H]$ glutamate released during perfusion of the tissue homogenate with 0.5 mM TMT was measured. At tissue concentrations from 2 - 6% the fractional release of transmitter was in the same range.

BRAIN REGION

The effect of TMT on transmitter release is here demonstrated on hippocampal homogenate. Similar results were obtained by using homogenate from neostriatum.

HIGH AFFINITY UPTAKE

The high affinity uptake of [^{3}H] glutamate into synaptosomes was significantly reduced (20 - 30%) with TMT concentrations from 50 µM to 1 mM in the incubation mixture (table 1). At 5 mM TMT the reduction was enhanced to 60%. The high affinity uptake of [^{3}H] GABA was reduced by 50 - 85% at TMT-concentrations from 0.5 mM to 5 mM.

RECEPTOR BINDING

The receptor binding of [^{3}H] glutamate and [^{3}H] GABA was inhibited by TMT (Table 1). At a ligand concentration of 100 nM the binding of [^{3}H] glutamate was reduced by 25% in a medium with 50 µM TMT compared to TMT-free controls. The binding of [^{3}H] GABA was not significantly altered at this TMT concentration and a 50 nM ligand concentration. 100 mM and higher concentrations of TMT produced a large inhibition (50 - 90%) of binding of both ligands. Figure 2 shows the saturation of the glutamate and GABA receptors at different TMT-concentrations. The effect of the tin-compound seemed to be a reduction in the number of sites (Bmax) for glutamate binding (Table 6) while Bmax for GABA binding and affinity constants (Kd) for both ligands were not significantly altered.

DISCUSSION

The release of L-[^{3}H] glutamic acid and D-[^{3}H] aspartic acid from synaptosomes have been shown to be enhanced by TMT at concentrations from 50 µM to 1 mM, while the release of [^{3}H] GABA was not significantly changed. The enhanced release of glutamate produced by

TMT has been shown to be calcium dependent, suggesting a synaptic release. High affinity uptake of transmitter is inhibited at concentrations from 50 mM to 5 mM for [^{3}H] glutamate and from 500 mM to 5 mM for [^{3}H] GABA. Binding of glutamate and GABA to synaptic membranes was reduced by concentrations of TMT from 50 mM to 1 mM (Table 1, Figure 2).

TMT has been reported to produce neuronal necrosis in the hippocampal formation after intraperitoneal administration of two doses of 4 mg/kg body weight to rats (Brown et al, 1979). The amount of TMT in the hippocampus after injection of the neurotoxic dose has been measured to 9.1 nmol/g wet weight (Aldrich, personal communication). The lowest concentration of TMT reported here to produce an interuption of neurotransmission (50 mM = 50 nmol/ml) in vitro is about five times higher than in the in vivo situation. This consentration is also in the same range as the concentration (3.5 mg/ml= 17.5 nmol/ml) required to depress the electric activity of the hippocampal neurons in vitro (Allen and Fonnum, 1984).[1]

The TMT stimulated release of glutamate was much smaller but had the same characteristics of the K^+ stimulated release in that it was Ca^{++}-dependent and TTX independent. The induction of release by TMT seems to be spesific for glutamate, since neither GABA (Table 1) nor acetylcholine release (Morot-Gaudry, 1984) is induced by TMT. The release of acetylcholine was inhibited by TMT during depolarization (Morot-Gaudry, 1984) when tested at a much higher TMT concentration, namely from 0.5 to 3.5 mM. The effect of TMT on acetylcholine and glutamate is therefore probably quite different.

Organotincompounds have been shown to induce several effects on membrane systems. They are associated with Cl^-/OH^- exchanges in mitochondria (Selwyn et al., 1970), erythrocytes and liposomes (Selwyn et al., 1970; Motais et al., 1977; Wieth and Tosteson, 1979) and in pancreatic β-cells (Pace and Tarvin, 1983). This exchange could produce a change in the internal and external Cl^--concentration. A reduction in the extracellular Cl^--concentration has been shown to

induce a glutamate release in vitro similiar to that induced by high potassium concentration (Hardy et al., 1984; Naalsund and Fonnum, 1986). This release takes place even at low changes in Cl^- concentration (Naalsund and Fonnum, 1986). A TMT induced chronic increased efflux of glutamate might be due to an alteration in external Cl^- concentration. Reduction in chloride concentration do not have the same stimulatory effect on the release of the inhibitory

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[1]According to the authors the actual concentrations used in these experiments are given in the discussion section of the paper. An error in the calculations has produced disagreement between molar and µg-concentrations.

transmitter GABA. (Naalsund and Fonnum, 1986). The lack of effect of TMT on GABA release supports a theory on Cl^- ions as mediators of TMT induced release. Measurements on anion exchanges in synaptosomes in the presence of TMT are at present lacking.

Our results on inhibition of uptake of GABA are in agreement with Doctor et al. (1982), who reported a 50% inhibition of GABA uptake in mouse forebrain synaptosomes at a TMT concentration of 75 µM. We found an inhibition of uptake of both GABA and glutamate with a slightly lower sensitivity of the GABA uptake to the TMT exposure than reprorted by Doctor et al.

The binding of glutamate and GABA to CNS membranes has been described by a number of different methods (for review see Foster and Fagg, 1984). The values for the equilibrium dissociation constant (Kd) and binding capasity (Bmax) in the litterature varies widely. We have measured the binding to a spesific population (saturable) of high to medium affinity binding sites (Kd < 500 nM) in a preparation of crude synaptic membranes. Na^+-ions were not present in the incubation mixture to exclude binding to high affinity uptake sites. The ability of TMT to inhibit the binding of transmitter and reduce the Bmax should reflect an interuption with the structural or chemical composition of the binding sites. The postsynaptic effect of TMT is in general agreement with the blocking of neurotransmission found by Allen and Fonnum (1984). According to Allen and Fonnum (1984) TMT exposure leads to a reduction in the number of action potensials produced by the CA1 pyramidal cells in response to stimulation of the Schaffer collaterals.

In conclusion our in vitro studies show a spesific effect of TMT on the release of glutamate possibly due to anion exchange, and an effect of TMT on both glutamate and GABA receptor sites. It is not possible at the present stage to say which effect is linked to the neuronal damage in the hippocampus. It is well established that glutamate is an excitotoxic amino acid (Olney et al., 1971), however the effect on the postsynaptic receptor might decrease such an excitotoxic effect.

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RFFERENCES

Aleu FP, Katzman R, Terry RD. Fine structure and electrolyte analysis of cerebral edema induced by alkyltin intoxication. J Neuropathol Exp Neurol 1963; 22:403-414.

Allen CN and Fonnum F. Trimethyltin inhibits the activity of hippocampal neurons recorded in vitro. Neurotoxicology 1984; 5(4): 23-30.

Barnes JM and Stoner HB. The toxicology of tin compounds. Pharmacol Rewies 1959; 11:211-231.

Baudry M and Lynch G. Regulation of hippocampal glutamate receptors: Evidence for the involvement of a calcium-activated protease. Proc Natl Acad Sci 1980; 77 (4):2298-2302.

Bouldin TW, Goines ND, Bagnell CR, Krigman MR. Pathogenesis of trimethyltin neuronal toxicity: Ultrastructural and cytochemical observations. Am J Pathol 1981; 104:237-249.

Brown AW, Aldridge WN, Street BW, Verschoyle RD. The behavioral and neuropathological sequelae of intoxication by trimethyltin compounds in the rat. Am J Pathol 1979; 97:59-82.

Catterall WA. The molecular basis of neuronal excitability. Science 1984; 223 (4637):653-661.

Doctor SC, Costa LG, Kendall DA, Murphy SD. Trimethyltin inhibits uptake of neurotransmitters into mouse forebrain synaptosomes. Toxicology 1982; 25:213-221.

Dyer RS, Deshields TL, Wonderlin WF. Trimethyltin-induced changes in gross morphology of the hippocampus. Neurobehav Toxicol Teratol 1982; 4:141-147.

Environmental Protection Agency. The manufacture and use of selected alkyltin compounds (EPA Report no. 56016-76-011) 1976; pp. 20-21.

Fonnum F, Lund-Karlsen R, Malthe-Sørenssen D, Sterri S, Walaas I. High affinity transport systems and their role in transmitter action. in: The Cell Surface and Neuronal Function, Cotman CW, Poste G, Nicholson GL eds. Elsevier/North-Holland Biomedical Press,Amsterdam. 1980; pp 455-504.

Fonnum F. Glutamate: A neurotransmitter in mammalian brain. J.Neurochem 1984; 42 (1):1-11.

Foster AC and Fagg G. Acidic amino acid binding sites in mammalian neuronal membranes: Their characteristics and relationship to synaptic receptors. Brain Res Rev 1984; 7:103-164.

Gray EG and Whittaker VP. The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. J Anat 1962; 96(1):79-88.

Hammerstad JP, Cawthon ML, Lytle CR. Release of [^{3}H]GABA from in vitro preparations: Comparison of the effect of DABA and β-alanine on the K^+ and protoveratrine stimulated release of [^{3}H]GABA from brain slices and synaptosomes. J Neurochem 1979; 32:195-202.

Hardy JA, Boakes RJ, Thomas DJE, Kidd AM, Edwardson JA, Virmani M, Turner J, Dodd PR. Release of aspartate and glutamate caused by chloride reduction in synaptosomal incubation media. J Neurochem 1984; 42(3):875-877.

Hill DR, Bowery NG, Hudson AL. Inhibition of GABAb receptorbinding by guanyl nucleotids. J Neurochem 1984; 42(3):652-657.

Lowry OH, Rosebrough NJ, Farr L, Randell RJ. Protein determination with the folin phenol reagent. J Biol Chem 1951; 193:265-275.

Morot-Gaudry Y. Inhibition by trimethyl-tin, probenecid and phenylglyoxal on depolarization-induced acetylcholine release in torpedo synaptosomes. Neurochemistry International 1984; 6(4): 553-561.

Motais R, Cousin JL,Sola F. The chloride transport induced by trialkyl-tin compounds across erythrocyte membrane. Biochimica et Biophysica Acta 1977; 467:357-363.

Naalsund LU and Fonnum F. Differences in anionic dependence of the synaptic efflux of D-aspartic acid and g-aminobutyric acid. J Neurochem 1986; in press.

Naalsund LU, Allen CN, Fonnum F. Changes in neurobiological parameters in the hippocampus after exposure to trimethyltin. Neurotoxicology 1985; 6(3):145-158.

Narahashi T. Pharmacology of nerve membrane sodium channels. Current topics in Membranes and Transport 1984; 22:483-516.

Olney JW, Ho OL, Rhee V. Cytotoxic effects of acidic and sulfur containing amino acids of the infant mouse central nervous system. Exp Brain Res 1971; 14:61.

Pace CS and Tarvin JT. pH modulation of glucose-induced electrical activity in b-cells: Involvement of Na/H and HCO_3/Cl antiporters. J Membrane Biol 1983; 73:39-49.

Selwyn MJ, Dawson AP, Stockdale M, Gains N. Chloride-hydroxide exchange across mitochondrial, erythrocyte and artificial lipid membranes mediated by trialkyl- and triphenyltin compounds. Eur J Biochem 1970; 14:120-126.

Watanabe I. Organotin (Triethyltin). In: Experimental and Clinical Neurotoxicology, PS Spencer and HJ Schaumburg,eds. Williams and Wilkins, Baltimore/London, 1980; pp 545-557.

Wieth JO and Tosteson MT. Organotin-mediated exchange diffusion of anions in human red cells. J Gen Physiol 1979; 73:765-788.

TABLE 1
THE EFFECT OF DIFFERENT TRIMETHYLTIN-CONCENTRATIONS ON THREE NEUROTRANSMITTER PARAMETERS: SPONTANOUS RELEASE, HIGH AFFINITY UPTAKE AND RECEPTOR BINDING.

Trimethyltin concentration	0.01 mM	0.05 mM	0.1 mM	0.5 mM	1 mM	5 mM	Activities in TMT-free controls, pmol/mg prot	
Induced release:							5mM K^+ per 10 min	50 mM K^+ per 10 min
^{3}H-Glutamate	105 ± 1(6)	120 ±2(11)*	137 ±8(14)*	144 ±4 (7)*	131 ±3 (9)*		59 ± 4	117 ± 7
^{3}H-D-Asp				215 ±32(4)*			52 ± 9	331 ± 11
^{3}H-GABA	105 ± 2(7)	108 ±1 (6)	115 ±2(16)	113 ±2 (5)	109 ±1 (9)		40 ±10	70 ± 16
HA-uptake,								
^{3}H-Glutamate	83 ± 1(4)	70 ±2 (4)*	75 ±3 (7)*	71 ±7 (4)*	70 ±4(11)*	39 ±4(7)*	0.46±0.16	
^{3}H-GABA	102 ±5 (8)	92 ±4 (8)	94 ±4(12)	51 ±2 (8)*	30 ±4(12)*	15 ±5(4)*	0.07±0.009	
Receptor binding,								
^{3}H-Glutamate	92 ±3(7)	75 ±4 (7)*	53 ±4 (7)*	17 ±5 (7)*	13 ±6 (7)*		216 ± 25	
^{3}H-GABA	68 ±13(3)	69 ±28(3)	36 ±10(3)*	22 ±10(3)*	26 ±12(3)*		1.27±0.11	

HA uptake and receptor binding are expressed in per cent of trimethyltin free controls, and are presented as mean ± SEM (number of experiments). Fractional release is expressed in per cent of the fractional release immidately before TMT perfusion. Ligand concentrations in binding studies were 100 nM for [^{3}H] glutamate and 50 nM for [^{3}H] GABA, and 70 nM for both ligands in high affinity uptake studies. *Indicates values significantly different from TMT-free controls (Dunnet's test, $p < 0.05$).

TABLE 2

THE EFFECT OF TRIMETHYLTIN ON THE BINDING CHARACTERISTICS OF GLUTAMATE AND GABA

LIGAND	TRIMETHYLTIN CONC	Bmax (pmol)	Kd (nmol)
[^{3}H] glutamate	0	136 ± 9	503 ± 46
	10 µM	99 ± 5*	415 ± 29
	100 µM	22 ± 1*	178 ± 40
	1 mM	2 ± .5*	279 ± 103

LIGAND	TRIMETHYLTIN CONC	Bmax (fmol)	Kd (nmol)
[^{3}H] GABA	0	335 ± 28	83 ± 17
	10 µM	205 ± 18	44 ± 6
	100 µM	240 ± 41	67 ± 16
	1 mM	179 ± 22	83 ± 17

Dissosiation constants (Kd) and maximum number of binding sites (Bmax) for the binding of glutamate and GABA in the presence of different concentrations of trimethyltin. The values were determined by linear regression analysis on a computer of the Scatchard plots derived from mean values of four assays in each of three saturation experiments. Ligand concentraitons in saturation studies were between 10 nM and 500 nM. Calculations are made according to the assumption that one type of binding sites is dominating in this concentration range. *Significantly different from TMT-free controls, $p < 0.05$ (Dunnett's test).

FIGURE 1 TIME COURSE AND INDEPENDENCE OF TRANSMITTER RELEASE AFTER STIMULATION WITH K^+ AND TMT.

Time course of release of L-[^{3}H] glutamate from hippocampal tissue in homogenate, induced by sequential stimulation with: A. 50 mM potassium (K^+), B. 0.1 mM trimethyltin (TMT). C and D shows that a previous stimulation with potassium does not influence the TMT-induced rlease and vice versa. The results are expressed as fractional release of tritiated transmitter in ten minutes during and after a four minutes perfusion with the indicated stimuli. Results are mean of three to six experiments. Frror bars represent SEM.

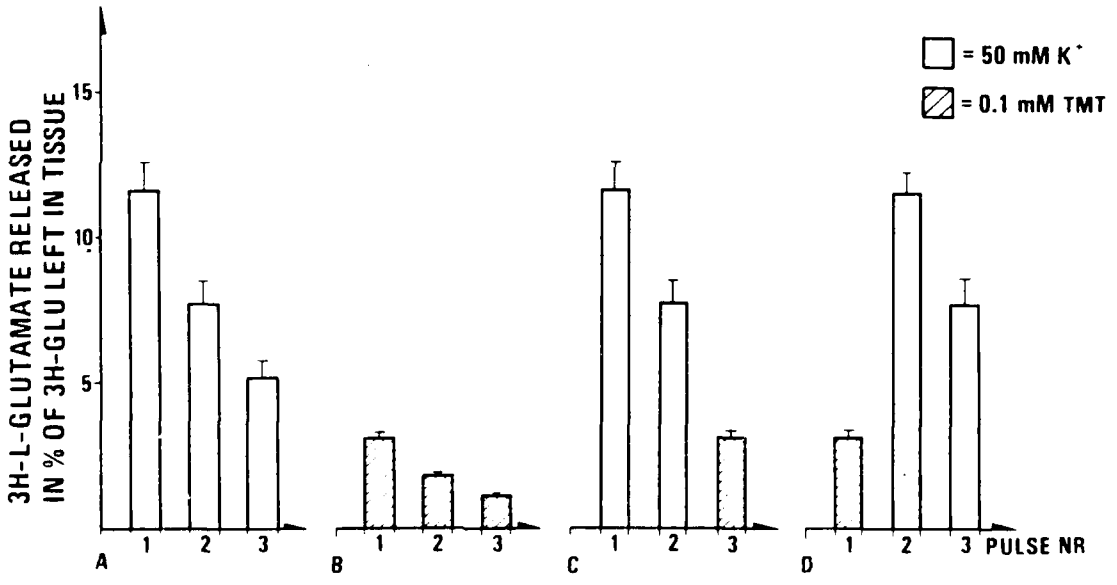
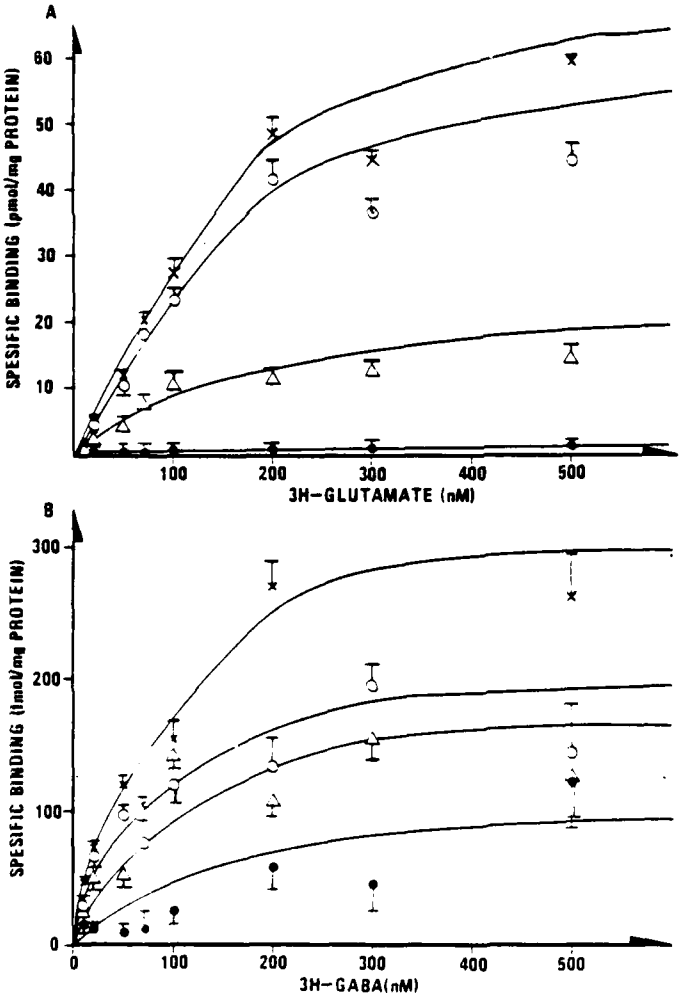


FIGURE 2. THE EFFECT OF TRIMETHYLTIN ON THE SATURATION OF GLUTAMATE AND GABA RECEPTORS.

Saturation plots of A. L-[^{3}H] glutamate and B. [^{3}H] GABA binding. Ligand concentrations between 10 nM and 500 nM were used in saturation studies. Each point represent spesific binding (the average of four assays in each of three experiments minus the measured binding in the presence of 1 mM unlabelled ligand), bars indicating SEM. x-x = TMT-free control; o-o = 10 µM TMT, Δ-Δ = 100 µM TMT, ●-● = 1 mM TMT.



DIFFERENCES IN ANIONIC DEPENDENCE OF THE SYNAPTIC EFFLUX OF D-ASPARTIC ACID AND γ-AMINO BUTYRIC ACID.

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ABSTRACT

The synaptosomal efflux of D-aspartate and GABA induced by a substitution of the Cl^--ions with propionate in the incubation medium were measured in preparations of hippocampal tissue homogenates. The efflux of aspartate was significantly higher than spontanous efflux at 125 mM Cl^- (normal = 144 mM) and was increased with decreasing Cl^--concentration. GABA efflux was much less sensitive to a reduction in Cl^--concentration than D-aspartate. The efflux was Ca^{2+}-dependent in both cases.

Key words: GABA, D-apartate, transmitters, chloride, efflux.

Running title: Anion changes induce transmitter efflux

INTRODUCTION

The synaptic efflux of endogenous transmitters or exogenously labelled transmitters and analogues have been studied by several different depolarization methods (reviews: Szerb, 1983, Fonnum, 1984). The transmitter amino acids are also secreted by depolarization of glial cell preparations, but this efflux can be separated from the synaptic efflux by its lack of Ca^{2+}-dependence (Sellström and Hamberger, 1977).

It is well established that a sudden change in the external $[K^+]$ or $[Cl^-]$ will depolarize the membrane of a muscle fibre (Hodgkin and Horowicz, 1960). Stimulation of synaptosomal release by high K^+-concentration is a freaquently used tool in neurochemical research. Recently it has been shown that a severe reduction in the Cl^--concentration in the perfusion medium was accompanied by an efflux of the transmitter amino acids glutamate and aspartate (Hardy et al., 1984). We are interested in studying the differences in efflux mechanisms for different transmitters.

The neurotoxic agent trimethyltin has been shown to induce the efflux of glutamate, but has no effect on the efflux of GABA (Naalsund and Fonnum, submitted). Trimethyltin is able to induce Cl^-/OH^- exchanges across cellular membranes (Motais et al., 1977). It has therefore been suggested that changes in Cl^-equilibrium could explain the observed effects on efflux. If trimethyltin is able to produce a change in the Cl^--equilibrium in the nerve terminal this could probably result in transmitter efflux.

GABA is known to act as a Cl^--ionophore (McBurney and Barker, 1978). We have therefore suggested that the reaction of the GABAergic nerveterminal to changes in extracellular Cl^--concentration might be different from that of the glutamergic terminals. If this is the case, it would give an explanation for the selective effect of trimethyltin on glutamergic neurons. This could also represent a basic difference

between the exicitatory glutamergic and inhibitory GABAergic neurons which could be helpful during discussions on different vulnerability to neurobiological tools. In this report we present the results of a comparative study of the efflux of D-aspartate and GABA during pulses of low Cl^--concentration.

METHODS

Tissue homogenates were prepared from the hippocampus dissected from the brain of young adult male Wistar rats (150-200 g). The hippocampi were homogenized in cold sucrose, 0.32 M, to give a protein concentration of 6 mg/ml. Efflux of D-[2,3-^{3}H] aspartic acid and [2,3-^{3}H(N)] GABA was measured by a modification of the method described by Levi et al., 1982. Briefly 2 µl homogenate containing about 12 µg protein was absorbed to a small piece of millipore filter. The filter was placed in a small tissue culture dish and 2 ml normal wash buffer (1st row table 1) was added. After preincubation at 25°C for 15 min the synaptosome pools were labelled with D-[^{3}H] aspartic acid or [^{3}H] GABA by incubation in the wash buffer containing 5 µM labelled transmitter (10-30 Ci/mmol) for 20 minutes at 25°C. The filters were washed four times in Krebs buffer and then placed in a small perfusion chamber made of two disposable plastic tips. The chambers were continously perfused with buffer at a constant flow of 0.4 ml/min. The perfusate was collected in fractions of 0.8 ml in counting vials. After a washperiod of 30 minutes the collection of fractions was initiated. An additional wash period of 20 minutes was followed by a 4 minutes perfusion period with one of the buffers numbered 2-7. The chambers were then washed with buffer 1 for 16 minutes before the experiment was terminated. To each fraction of the perfusate were added 10 ml Opti-fluor (Packard) and the radioactivities were counted. The filters were dissolved in Filtercount (Packard) and the rest activity measured.

For measurments of the Ca^{2+}-dependence the buffers were substituted with "low-Ca^{2+}"-buffers containing 0.1 mM Ca^{2+}, (nr. 7-9) before the

collection of the fractions started. All the GABA-efflux experiments were performed in the presence of 0.1 mM of the GABA-transaminase inhibitor, amino oxyacetic acid.

High affinity uptake of transmitter was measured in the presence of either 70 nM D-[^{3}H] aspartic acid or 70 nM [^{3}H] GABA. Crude homogenates were diluted with Krebs buffer containing either 150 mM Cl^- or 50 mM Cl^- and 100 mM propionate. (For details on the method see Fonnum et al, 1980).

RESULTS AND DISCUSSION

The present study shows that the efflux of the glutamate analogue D-aspartic acid is much more sensitive than the efflux of the inhibitory transmitter GABA to reduction in the Cl^--concentration. The efflux of D-apartic acid was inversely dependent on the concentration of Cl^- and significantly higher than normal spontanous efflux at all the Cl^--concentrations tested (Table 2). A reduction in the $[Cl^-]$ to 6 mM gave an efflux which was four times the efflux stimulated by 50 mM K^+. The GABA efflux during exposure to low Cl^- was approximately 50% lower than the D-aspartate efflux at Cl^--concentrations from 6 mM to 50 mM and not significantly different from the normal spontanous efflux for chloride concentratoins above 100 mM.

The Cl^--ions were substituted with propionate, which does not pass through the Cl^--channel according to Eccles (1964). This substitution should therefor produce a change in the relation between intracellular and extracellular Cl^--concentration. According to the Goldman constant-field equation, which states that the membrane potential is dependent upon the distribution of K^+, Na^+ and Cl^-, this will create a shift in the membrane potential towards depolarization, and thereby result in an efflux of transmitter. The duration of the depolarization and hence the amount of transmitter released will depend on diffusion and the cell's ability to transport Cl^- out of the cell to restore an equilibrium. This hypothesis was supported by our results which are an extension of the previous findings by Hardy et al. (1984).

It is well known that the synaptosomal high affinity uptake of GABA is dependent upon a high concentration of extracellular Cl^- (Kanner, 1978), while the uptake of glutamate is less dependent on high $[Cl^-]$ (Kanner and Sharon, 1978). The effect on reuptake of transmitter produced by changes in the anion composition was measured for both D-$[^3H]$ aspartic acid and $[^3H]$ GABA. Uptake of D-$[^3H]$ aspartic acid was reduced by 20% ± 2% (mean ± SEM, n=4) after replacing 100 mM Cl^- with propionate, while $[^3H]$ GABA uptake showed 24% ± 4% (mean ± SEM, n=6) reduction. With the same ionic composition the observed increase in efflux was 300% for D-aspartic acid and 200% for GABA. These results suggests that the measured efflux was not merely due to a reversal of the uptake mechanism that could possibly have been caused by the changes in ionic gradients. It also shows that the observed increase in tritiated transmitter in the perfusate was mainly due to an increased efflux rather than to a reduced reuptake. Additional evidence for this is provided by earlier reports stating that continous perfusion at the rate used here (0.4 ml/min) minimizes reuptake of the excreted transmitter (Raiteri et al, 1975).

The measured efflux was considered to be of synaptosomal origin. Glial cell uptake and release of transmitter are very labile to mechanical disruption such as homogenization (Fonnum, 1984). Glial release is therefor normally not a major part of the efflux measured by the method used here. This was verified by a demonstration of the Ca^{2+}-dependence of the efflux. D-Aspartic-acid efflux induced by 50 mM K^+ was reduced by 40% $\pm$ 2% (mean ± SEM, n=4) by a reduction of calcium in the medium from 2 mM to 0.1 mM. The efflux induced by 50 mM Cl^- was reduced by 46% $\pm$ 2% (mean ± SEM, n=4) in the low-calcium medium. GABA-efflux was reduced by 67% ± 5% (mean ± SEM, n=4) and 71% ± 4% (mean ± SEM, n=4) for high potassium- and low chloride-induced efflux respectively. The high Ca^{2+}-dependence of the chloride stimulated efflux and the potassium stimulated efflux suggests that large parts of the measured effluxes were associated with a depolarization of the synaptosomal membrane.

Cl^--ions are essensial for ATP-dependent proton transport across the membranes of catecholamine storage vesicles (Pollard et al, 1979) and

the microsomal vesicles in plant cells (Bennet and Spanswick, 1983). Reduction in $[Cl^-]$ will reduce proton transport and thereby collapse the pH-gradient across these vesicle membranes, resulting in leakage of the stored molecules. Some evidence exists for the storage of glutamate in synaptosomal vesicles (Naito and Ueda, 1985), while the existence of a vesicular storage of GABA remains unclear. The possibility of a vesicular storage of the transmitter amino acids raises the question of whether the observed efflux reported in the present paper is the result of a change in the pH-gradient across vesicle membranes. Changes in intracellular $[Cl^-]$ is very slow following an extracellular reduction in $[Cl^-]$ (Kelly et al, 1969), and could not produce a pH-change resulting in an immediate efflux of transmitter after a change in the anion composition of the perfusion buffer. Diffusion of propionate in the free acid form across the cell membrane is known to take place at a slightly faster rate than the Cl^- diffusion (Carvalho and Carvalho, 1979). This may represent a cause of a reduction in the intracellular pH resulting in vesicular leakage of transmitter, although this diffusion also seems to need about 1 - 2 minutes before equilibrium is achieved (Carvalho and Carvalho, 1979) and hence should give a lag time before any transmitter efflux.

GABA is known to increase the membrane permeability to Cl^--ions (McBurney and Barker, 1978). A possible explanation for the lack of effect of a 45 mM reduction in the Cl^--concentration on the measured GABA efflux could possibly be due to the GABA-ergic neuron having a greater ability to restore a transmembrane equilibrium of Cl^-. The presence of presynaptic GABA receptors may increase the synaptosome's ability to regulate GABA efflux (Mitchell and Martin, 1978).

Our results showing that a 20-40 mM reduction in Cl^--concentration produced an efflux of D-aspartic acid which was twice the amount of the spontanous efflux, while the same Cl^--change had no effect on the GABA efflux, is an interesting observation, which should be taken into account in studies of drugs that may act on Cl^--channels.

REFERENCES

Bennet A.B. and Spanswick R.M. (1983) Optical measurements of pH and in corn root membrane vesicles: kinetic analysis of Cl^- effects on a proton-translocating ATPase. J. Membr. Biol. 71, 95-107.

Carvalho C.A.M. and Carvahlo A.P. (1979) Effect of temperature and ionophores on the permeability of synaptosomes. J. Neurochem. 33, 309-317.

Eccles J.C. (1964) The ionic mechanism generating the inhibitory post-synaptic potential, in Physiology of Synapses, pp. 173-188. Springer-Verlag, Berlin.

Fonnum F. (1984) Glutamate: a neurotransmitter in mammalian brain. J.Neurochem. 42, 1-11.

Fonnum F., Lund-Karlsen R., Malthe-Sørenssen D., Sterri S., and Walaas I. (1980) High affinity transport systems and their role in transmitter action. in: The Cell surface and Neuronal Function, (Cotman C.W., Poste G., and Nicholson G.L., eds), pp. 455-504. Elsevier/North-Holland Biomedical Press, Amsterdam.

Hardy J.A., Boakes R.J., Thomas D.J.E., Kidd A.M., Edwardson J.A., Virmani M., Turner J., and Dodd P.R. (1984) Release of aspartate and glutamate in synaptosomal incubation media. J. Neurochem. 42, 875-877.

Hodgkin A.L. and Horowicz P. (1960) The effect of sudden changes in ionic concentrations on the membrane potential of single muscle fibres. J. Physiol. 153, 370-385.

Kanner B.I. (1978) Active transport of -aminobutyric acid by membrane vesicles isolated from rat brain. Biochemistry 17, 1207-1211.

Kanner B.I. and Sharon I. (1978) Active transport of L-glutamate by membrane vesicles isolated from rat brain. Biochemistry 19, 3949-3953.

Kelly J.S., Krnjevic K., Morris M.E., and Yim G.K.W. (1969) Anionic permeability of cortical neurons. Expl. Brain Res. 7, 11-31.

Levi G., Gordon R.D., Gallo V., Wilkin G.P., and Balázs R. (1982) Putative acidic amino acid transmitters in the cerebellum I. depolarization-induced release. Brain Res. 239, 425-445.

McBurney R.N. and Barker J.L. (1978) GABA-induced conductance fluctuations in cultured spinal neurons. Nature 274, 596-597.

Mitchell P.R. and Martin I.L. (1978) Is GABA release modulated by presynaptic receptors. Nature 274, 904-905.

Motais R., Cousin J.L., and Sola F. (1977) The chloride transport induced by trialkyl-tin compounds across erythrocyte membrane. Biochim. Biophys. Acta 467, 357-363.

Naalsund L.U., Allan C.N., and Fonnum F. (1985) Changes in neurobiological parameters in the hippocampus after exposure to trimethyltin. Neurotoxicology 6, 145-158.

Naalsund L.U., and Fonnum F. The effect of trimethyltin on three glutamergic and GABAergic transmitter parameters in vitro: high affinity uptake, release and receptorbinding. (submitted to Neurotoxicology)

Naito S. and Ueda T. (1985) Characterization of glutamate uptake into synaptic vesicles. J. Neurochem. 44, 99-109.

Pollard H.B., Shindo H., Creutz C.E., Pazoles C.J., and Cohen J.S. (1979) Internal pH and state of ATP in adrenergic chromaffin granules determined by ^{31}P nuclear megnetic resonance spectroscopy. J. Biol. Chem. 254, 1170-1177.

Raiteri M., Federico R., Coletti A., and Levi G. (1975) Release and exchange studies relating to the synaptosomal uptake of GABA. J. Neurochem. 24, 1243-1250.

Sellström Å. and Hamberger A. (1977) Potassium-stimulated -amino-butyric acid release from neurons and glia. Brain Res. 119, 189-198.

Szerb J.C. (1983) Mechanisms of GABA release, in Glutamine, Glutamate, and GABA in the Central Nervous System (Hertz L., Kvamme E., McGeer E.G., and Schousboe A., eds), pp. 457-472. Alan R. Liss, Inc., New York.

TABLE 1. COMPOSITION OF MEDIA (mM)

Number	1	2	3	4	5	6	7	8	9	10
Name:	normal wash	125 Cl^-	100 Cl^-	50 Cl^-	25 Cl^-	6 Cl^-	50 K^+	wash low Ca^{2+}	50 Cl^- low Ca^{2+}	50 K^+ low Ca^{2+}
Na^+	137.6	137.6	137.6	137.6	137.6	137.6	85.6	137.6	137.6	85.6
K^+	7.4	7.4	7.4	7.4	7.4	7.4	51.8	7.4	7.4	51.8
Mg^{2+}	1	1	1	1	1	1	1	10	10	10
Ca^{2+}	2	2	2	2	2	2	2	0.1	0.1	0.1
SO_4^{2-}	1	1	1	1	1	1	1	10	10	10
$H_2PO_4^-$	5	5	5	5	5	5	5	5	5	5
Cl^-	144	125	100	50	25	6.4	146.4	144	50	146.4
Propionate	-	18	44	94	119	137.6	-	-	94	-
Glucose	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Hepes	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5

TABLE 2. RELEASE OF TRANSMITTER AMINO ACIDS

Buffer used to normal induce release wash		50 K^+	125 Cl^-	100 Cl^-	50 Cl^-	25 Cl^-	6 Cl^-
Preloaded amino acid:							
D-[^{3}H]aspartic acid	4 ± 0.7	10 ± 1*	3 ± 0.4*	5 ± 1*	13 ± 1*	15 ± 1*	36 ± 5*
[^{3}H] GABA	3 ± 0.4	9 ± 1*	0 ± 0	1 ± 0	7 ± 1*	6 ± 9*	18 ± 1*

Increase in amino acid release from rat hippocampal synaptosomes into the perfusion medium in per cent of total content of tritiated amino acid in tissue after replacement of the normal wash buffer (1st row) with a buffer with the indicated composition. Results are means ± SEM of eight experiments. *Increase is significant, $p < 0.01$ (Wilcoxon's two sample test). Potassium induced release is not significantly different for D-[^{3}H] aspartic acid and [^{3}H] GABA. All Cl^--concentrations tested induce a significantly lower release of [^{3}H] GABA than of D-[^{3}H] aspartic acid, $p < 0.01$ (Student's t-test).

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HIPPOCAMPAL EEG IN RATS AFTER CHRONIC TOLUENE INHALATION

by

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Running title: Toluene-induced changes in hippocampal EEG.

ABSTRACT

Rats were chronically implanted with bipolar electrodes in the hippocampal regions containing cells generating electric theta-activity. The animals were exposed to 500 ppm of toluene in inhalation chambers, for 8 or 16 hours per day for 5 days per week in 12 weeks. The hippocampal electric activity was recorded 48 hours after each weekly exposure, ensuring a minimal amount of toluene in the tissue during the recordings. The eight hours daily exposed group showed an initial period of increased frequency of the regular theta waves together with an increased incidence of theta activity after 1 - 2 weeks of exposure. In the sixteen hours daily exposed rats two weeks of toluene inhalation produced a significant reduction in the theta wave frequency. This change was also reached after eight weeks of exposure in the eight hours daily exposed group. At this moment the theta activity was frequently disrupted by short amplitude irregular waves, a phenomen which increased gradually throughout the rest of the exposure period. The average blood concentration of toluene was 16.7 mg/ml and 17.7 mg/ml and not significantly different for the eight and sixteen hours exposed groups respectively.

Key-words: Hippocampal EEG, Inhalation, Toluene.

INTRODUCTION

Neurophysiological tests allow relatively direct assessment of the functional integrity of specific neuronal systems. These tests also have the advantage that they can be carried out several times in the same animal, and give valuable information concerning the development of a brain dysfunction.

EEG as a screening test for solvent induced brain damage in humans has not been recomended because of a large variation in the normal EEG's. In a normal population 10 - 15% may have EEG abnormalities (Seppäläinen 1985). In animals however, the use of intracerebral implantation of electrodes in defined brain areas enables recordings with a high specificity and with a minimal interindividual variation.

Hippocampal EEG is characterized by a very prominent rhythm designated the theta rhythm, with an approximately sinusoidal pattern of electrical activity of different frequency ranges in various species (6-12 Hz in the rat). Theta rhythm can be recorded from the hippocampus of several mammalian species, it possess a strong biological potential (1-3 mV in amplitude), and can readily be recorded by electrodes properly implanted within the hippocampus. The theta rhytm is selectively present during spesific behaviors (Vertes 1982).

We have previously used hippocampal EEG to study the development of a degeneration process after injection of the well known neurotoxic substance, trimethyltin (Naalsund et al. 1985). This study showed that the hippocampal electric activity was a more sensitive parameter than histological and neurochemical parameters for early detection of damage.

Toluene has been reported to produce alterations in CNS excitability (Benignus 1981) and sensory function (Pryor et al. 1983a; 1983b; Dyer et al. 1984) in animal studies and in humans (Metric and Brenner 1982; Cooper et al. 1985). Abnormal electroencephalographic (EEG) activity

after toluene inhalation has been found in several cases after both abuse and occupational exposure (Andersen and Kaada 1953; Knox and Nelson 1966; Seppäläinen et al. 1980; Grasso et al. 1984), as well as in animal experiments (Takeuchi and Hisanaga 1977).

The present paper describes neurophysiologic studies designed to detect alterations in the hippocampal theta cells or their activating or inhibitory connections following exposure to 500 ppm toluene at various time intervals. This level is above the recomended TLV (threshold limit value) of 75 ppm for an 8 hr exposure (Norwegian Labour Inspection 1984), but is within the range of some short-term exposure situations (Benignus 1981) and far below the exposure level for "sniffers" (Benignus 1981).

MATERIALS AND METHODS

Surgery. The hippocampal EEG was recorded in male Wistar rats with chronically implanted electrodes. Initial body weights were between 300 and 400 g. Surgical preparation of the rats were performed under diazepam (5 mg/kg i.p.) and fluanizone (7.5 mg/kg s.c.) anesthesia. The rats were placed in a stereotaxic frame, and the skin and connective tissue on the skull were removed. Stainless steel screws were set in the skull to serve as anchors for the electrodes. The electrodes were made of a silverplated copperalloy conductor (127 mM diam.) insulated with teflon and each soldered to a male golden pin component (220-P02100 Bunker Ramo, Amphenol North America). The electrodes were twisted and cut so that one tip was 0.7 mm shorter than the other. Only the tips were bared of insulation and they were spaced 1.5 mm from one another. The electrodes were fixed to the anchors with dental cement.

Placement of such electrodes in the dorsal hippocampus with one in the subfield CA1 of the pyramidal cell layer and the other in the granular layer of fascia dentata (FD), permits the recording of clear and large amplitude theta activity either bipolarly or unipolarly (Sainsbury and

Bland 1981). One of the screws served as a zero level during unipolar recordings. The implantation coordinates were the following: 3.2 mm behind bregma, 2.0 mm lateral to the midline and 3.0 mm depth from the dura (fig. 1). The electrode pair was longitudinally oriented with the deepest electrode in posterior position.

Recording. After the surgery the rats were allowed ten days to recover before any recordings were taken. At least two recordings were carried out before the exposure period, and one recording per week during exposure time. The rats were allowed two days of recovery from exposure before recording at any time, ensuring no solvent to be present in the tissue (Pyykkö et al. 1977). During recording the animals were situated in cages (50 x 30 x 15 cm) without cover and connected to the graph (Grass Electroencephalograph model IIIc) through a female plug. The behavior of the rats were coded on the records throughout the test period. Each rat was tested for five to ten minutes, assuring that different behavior types were seen.

Exposure. Exposure to toluene was performed in an inhalation chamber, volume 300 litre, with constantly supply of vaporized toluene diluted to 500 ppm in the inlet air, and a ventilation of 3000 l per hour. The toluen concentration was measured continuously with a Miran IR spectrophotometer connected to a recorder. A second chamber was ventilated with fresh air for control experiments. Temperature and humidity in the animal room were controlled according to OECD guidlines (22 ± 3°C, 30-70% relative humidity).

The animals were divided into four groups: two groups spent eight hours per day in the toluene and control chamber, respectively, while the other two groups were kept in the chambers for 16 hours per day. The exposures were carried out for five days per week. The eight hours exposure was carried out during daylight hours and in an illuminated room, while the sixteen hours was performed during the dark hours and without illumination. This was done for practical reasons. The effect of the activity level of the animals on the uptake of toluene was

tested by blood-concentration measurements (gass-chromatography, headspace tecnique).

RESULTS

The daylight exposed group had an average blood-concentration of toluene of 16.7 ± 2.7 µg/ml (N=3), while for the dark-exposed group the concentration was 17.7 ± 1.8 µg/ml (N=3), measured at the end of the fifth exposure day. There was no statistical difference between the two groups.

Clear theta waves were recorded during type 1 behaviors (walking, rearing, changes in posture, head movements). Frequencies of the theta waves were estimated from at least five 1 sec segments of graph records from each brain half during walking or rearing. Average values of these estimates are presented as a function of exposure time in fig. 2. The animals exposed for eight hours per day show an initial increase in the frequency followed by a decline. The animals exposed for sixteen hours showed a faster and more pronounced decline in frequency. Ten days of toluene inhalation produced a significant reduction in the theta-wave frequency in the rats with longest exposure intervals, whereas forty days of exposure produced the same change in the eight hours exposed group.

Toluene exposure was terminated after 60 days of exposure. Two animals from each group were allowed a recovery period of one month before a last recording was performed. No increase in the theta wave frequency had occured during this recovery period, which indicate that the observed changes was irreversible. Neither of the control groups showed any change in frequency or quality of the theta activity during and after the treatment period.

Parallell to the changes in frequency of the theta waves, qualitative changes also occured. The first period of increased frequency was not

accompanied by any disruptions in the regular waves during type 1 behavior. During immobility, which is normally associated with irregular waves, however theta waves were in some instances seen in this period (fig. 3). The reduction in theta frequency was followed by a disruption of the regular waves. After 60 days of exposure regular theta activity were absent in the sixteen hours daily exposed group (fig. 4) while the eight hours daily exposed group had frequent disruptions with short amplitude waves during the theta-activity (fig. 3). Only slight improvement in the quality of the theta waves are seen after the one month recovery period.

DISCUSSION

Toluene inhalation produced disruptions and frequency changes in the hippocampal theta activity. After an initial increase in the frequency and an increase in the occurence of theta waves, there was a reduction in the frequency and a gradually increased disruption of the theta waves. The degree of changes were related to the length of the exposure period and to the daily exposure intervals. Sixteen hours per day with 500 ppm toluene produced frequency reduction after ten days, while eight hours daily exposure to the same concentration gave frequency reduction after forty days. Recovery for one month gave small qualitative improvements but no increase in frequency towards normal theta waves. The initial increase in frequency was only seen in the eight hours exposed group. It is likely that a frequency increase in the sixteen hours exposed group could have occured during the first five days of exposure and therefore have not been detected.

Previously it has been shown that very high doses of toluene (4000 ppm) in short exposure intervals (four hours) will reduce hippocampal theta wave frequency and simultanously give an increase in the theta component of the hippocampal EEG (Takeuchi and Hisanaga 1977). The present report confirm and extend the nature of these observations and suggest that also low concentrations (500 ppm) of toluene can induce such changes during chronic exposure.

In the rat there are only two rather well-defined behaviors during which theta activity is present, i.e. voluntary motion and paradoxical sleep (Winson 1974). This is not true for other species, eg. cats and rabbits, in which extensive behavioral observations have been made (Winson 1972). During ether or urethane anesthesia spontanous theta waves have been observed in the rat (Bland and Whishaw 1976). A similar anesthetic effect could possibly explain the observed theta activity during immobility in the toluene exposed rat.

Several nuclei at different levels of the neural axis from the brain stem to septum appear to be involved in hippocampal theta generation. With few exceptions, studies indicate that the medial pontine region of the reticular formation is the main source for hippocampal synchronization and that the median raphe nucleus is the brain stem source for hippocampal desynchronization (Vertes 1982). The communication between the brain stem and the hippocampus are mediated via the medial septum/diagonal band (Vertes 1982). Septal lesions can give complete loss of hippocampal theta-activity (Andersen et al. 1979). The effects of brain stem lesions on the hippocampal EEG are less straightforward, possibly because ascending brain stem systems are more diffusely organized than the septal- hippocampal system. Increased occurence of hippocampal theta, as were seen in the rats in a period during toluene exposure, may reflect damage to desynchronizing systems. Lesions in the median raphe nucleus and the lateral hypothalamus (Vertes 1982) shows the characteristics of damage to such pathways: the occurence of a lowfrequency theta (5.8 Hz) during immobility. Several synchronizing pathways maintain the theta activity (Vertes 1982), an interuption of one synchronizing system would therefore have the effect of slightly reducing the theta frequency but not abolishing theta (Vertes 1982). From these data it follows that our observations could be associated with both a damage to the desynchronizing and to the synchronizing pathways.

Increased frequency and improved quality of hippocampal theta waves during type 1 behavior is also reported in rats with severe motor deficits after surgically prepared cerebellar lesions (Myhrer and

Allen 1985). It is suggested that activation of compensatory mechanisms due to adoption of new behavioral strategies could be reflected in this way.

Hippocampal theta activity may be manipulated by systemic or iontophoretic application of pharmacologically active substances. A large number of drugs depress the theta rhythm of the hippocampus, among these are anesthetics, hypnotics, tranquilizers and related drugs (Stumpf 1965). In situ injection in the medial septal nucleus, of atropine or GABA, inhibits theta activity (Kolb and Whishaw 1977; Allen and Crawford 1984) and the same effect can also be achieved by blocking the excitatory action of glutamate in the hippocampus (Fontain et al. 1984). Cholinergic agents are known to produce theta waves, provided the septal area is intact (Stumpf 1965). The mechanism by which toluene can produce an irreversible depression of the hippocampal theta activity should be quite different from the acute membrane fluidizing action of organic solvents (Franks and Lieb 1978) for which the membranes seem to develope a tolerance (Chin and Goldstein 1977). According to Pyykkö et al. (1977) the toluene concentration in brain tissue is reduced to 0.1% of its level during exposure, 24 hours after termination of the exposure. This indicates that acute effects have not been measured in our experiments. In view of the results from surgical and pharmacological manipulations of the theta rhythm it is clear that the observed changes in hippocampal EEG after toluene inhalation could be the result of a toxic action on any part of the neural system generating or regulating the theta activity.

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REFERENCES

Allen, C.N. & I.L. Crawford: GABAergic agents in the medial septal nucleus affect hippocampal theta rhythm and acetylcholine utilization. Brain Res. 1984, 322, 261-267.

Andersen, P. & B.R. Kaada: The electroencephalogram in poisoning by lacquer thinner (butyl acetate and toluene). Acta pharmacol. et toxicol. 1953, 9, 125-130.

Andersen, P., H.B. Bland, T. Myhrer & P.A. Schwartzkroin: Septo-hippocampal pathway necessary for dentate theta production. Brain Res. 1979, 165, 13-22.

Benignus, V.A.: Health effects of toluene: A review. NeuroToxicology 1981, 2, 567-588.

Bland, B.H. & I.Q. Whishaw: Generators and topography of hippocampal theta (RSA) in the anaesthetized and freely moving rat. Brain Res. 1976, 118, 259-280.

Chin, J.H. & D.B. Goldstein: Drug tolerance in biomembranes: A spin label study of the effects of ethanol. Science, 1977, 196, 684-685.

Cooper, R., P. Newton & M. Reed: Neurophysiological signs of brain damage due to glue sniffing. Electroencephalogr.Clin.Neurophysiol. 1985, 60, 23-26.

Dyer, R.S., K.E. Muller, R. Janssen, C.N. Barton, W.K. Boyes & V.A. Benignus: Neurophysiological effects of 30 day chronic exposure to toluene in rats. Neurobehav.Toxicol.Teratol. 1984, 6, 363-368.

Fontani, G., F. Grazzi & M. Meucci: Cholinergic and glutaminergic control of hippocampal RSA during behavior in rabbits. Physiol. Behav. 1984, 33, 699-706.

Franks, N.P. & W.R. Lieb: Where do general anesthetics act? Nature, 1978, 274, 339-342.

Grasso, P., M. Sharratt, D.M. Davies & D. Irvine: Neurophysiological and psychological disorders and occupational exposure to organic solvents. Food and Chem. Toxicol. 1984, 22, 819-852.

Kolb, B. & I.Q. Whishaw: Effects of brain lesions and atropine on hippocampal and neocortical electroencephalograms in the rat. Exp. Neurol. 1977, 56, 1-22.

Knox, J.W. & J.R. Nelson: Permanent encephalopathy from toluene inhalation. N. Eng. J. Med. 1966, 275, 1494-1496.

Metrick, S.A. & R.P. Brenner: Abnormal brainstem auditory evoked potentials in chronic paint sniffers. Ann. Neurol. 1982, 12, 553-556.

Myhrer, T. & C.N. Allen: Hippocampal EEG changes in rats following cerebellar lesions. Physiol. Behav. 1985, 35, 377-382.

Naalsund, L.U., C.N. Allen & F. Fonnum: Changes in neurobiological parameters in the hippocampus after exposure to trimethyltin. NeuroToxicology, 1985, 6, 145-158.

Norwegian Labour Inspection: TLV-values for polutions in the working environment, 1984.

Paxinos, G. & C. Watson: The rat brain in stereotaxic coordinates. 1982, Academic Press, Sydney.

Pryor, G.T., J. Dickinson, R.A. Howd & C.S. Rebert: Neurobehavioral effects of subchronic exposure of weanling rats to toluene or hexane. Neurobehav. Toxicol. Teratol. 1983a, 5, 47-52.

Pryor, G.T., J. Dickinson, R.A. Howd & C.S. Rebert: Transient cognitive deficits and high-frequency hearing loss in weanling rats exposed to toluene. Neurobehav. Toxicol. Teratol. 1983b, 5,

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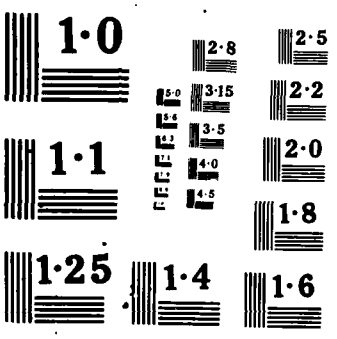


Pyykkö, K., H. Tähti & H. Vapaatalo: Toluene concentrations in various tissues of rats after inhalation and oral administration. Arch. Toxicol. 1977, 38, 169-176.

Sainsbury, R.S. & B.H. Bland: The effects of selective septal lesions on theta production in CA1 and the dentate gyrus of the hippocampus. Physiol. Behav., 1981, 26, 1097-1101.

Seppäläinen, A.M.: Neurophysiological aspects of the toxicity of organic solvents. Scand. J. Work Environ. Health 1985, 11, suppl.1, 61-64.

Seppäläinen, A.M., K. Lindström & T. Martelin: Neurophysiological and psychological picture of solvent poisoning. Am. J. Ind. Med. 1980, 1, 31-42.

Stumpf, C.: Drug action on the electrical activity of the hippocampus. Int. Rev. Neurobiol. 1965, 8, 77-138.

Takeuchi, Y. & N. Hisanaga: The neurotoxicity of toluene: EEG changes in rats exposed to various concentrations. Brit. J. Ind. Med. 1977, 34, 314-324.

Vertes, R.P.: Brain stem generation of the hippocampal EEG. Prog. Neuorobiol. 1982, 19, 159-186.

Winson, J.: Interspecies differences in the occurence of theta. Behav. Biol. 1972, 7, 479-487.

Winson, J.: Patterns of hippocampal theta rhythm in the freely moving rat. Electroenceph. Clin. Neurophysiol. 1974, 36, 291-301.

FIGURE 1.

Representative example of location of electrodes. The implantation coordinates were for the dentate electrode (FD): 4.7 mm behind bregma, 2.0 mm lateral to the midline and 3.7 mm below the dura, and for the pyramidal electrode (CA1): 3.2 mm, 2.0 mm and 3.0 mm given in the same order. The section shown is a frontal view 3.3 mm behind bregma (Paxinos and Watson, 1982).

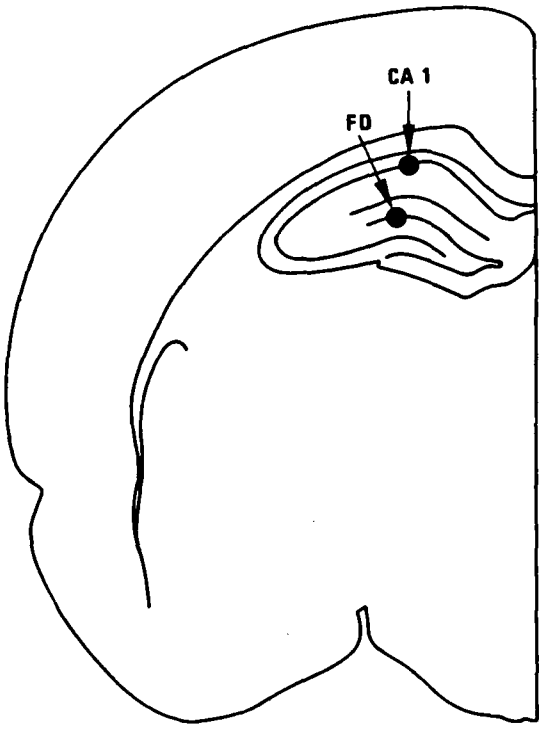


FIGURE 2.

Frequency of theta waves during walking or rearing after exposure to 500 ppm toluene for eight (x) or sixteen (o) hours per day, five days per week. Toluene inhalation was started at point zero on the horizontal axis, days without exposure are not counted in the exposure period (5 days = 1 week). Each point represent an average of three to eight animal records (one brain half give one record), and the bars indicate the standard error of mean. * Significant different from controls ($p<0.05$, Student's T-Test).

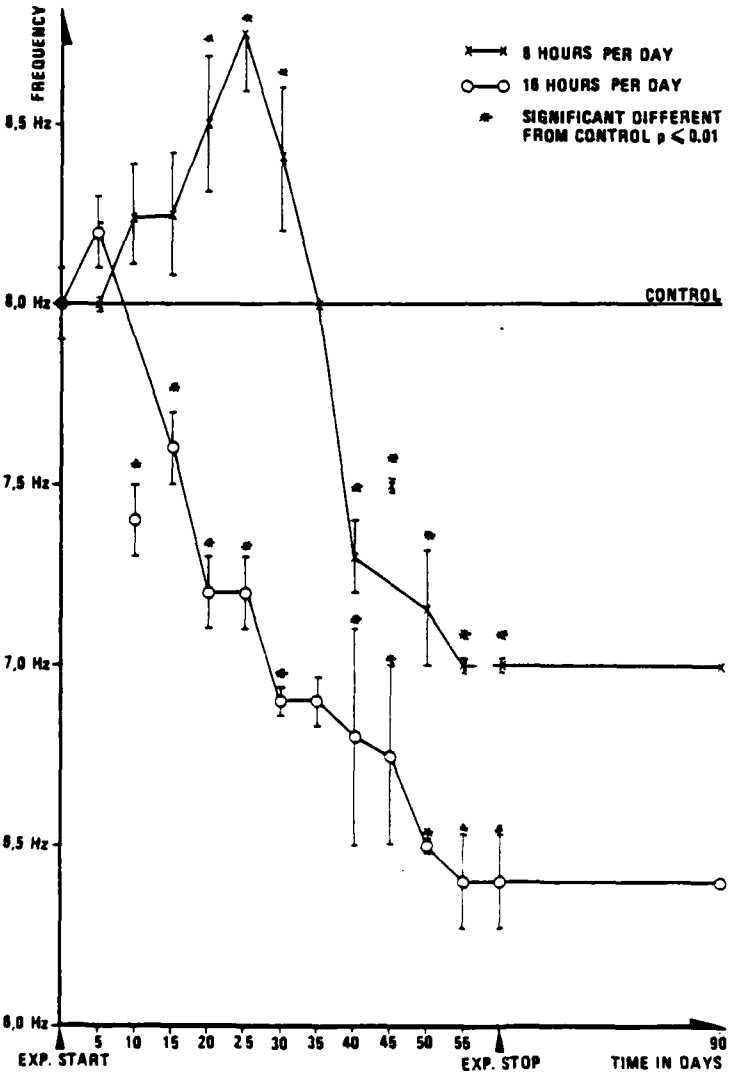


FIGURE 3.

Electric activity in the dorsal hippocampal formation before, during and after a 60 day period with toluene inhalation, 500 ppm for eight hours per day, five days per week. Days without exposure are not counted in the exposure period. The records are bipolar registrations from a single rat.

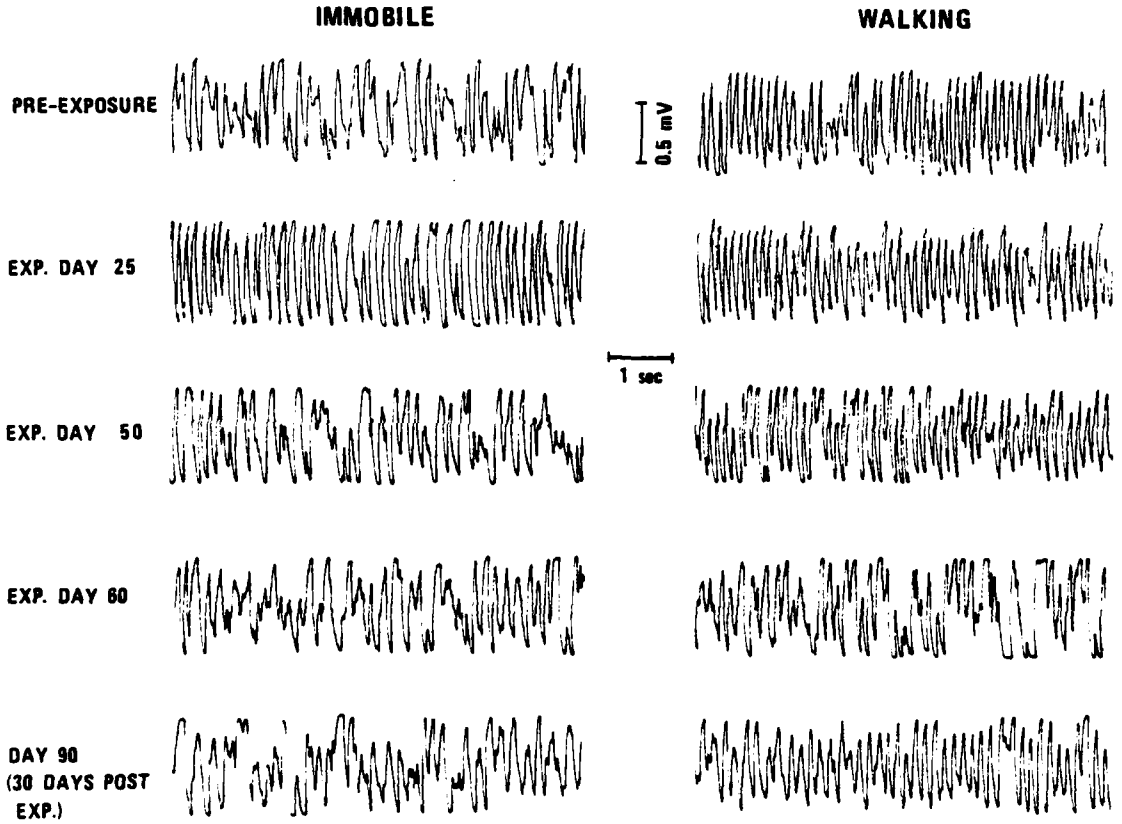
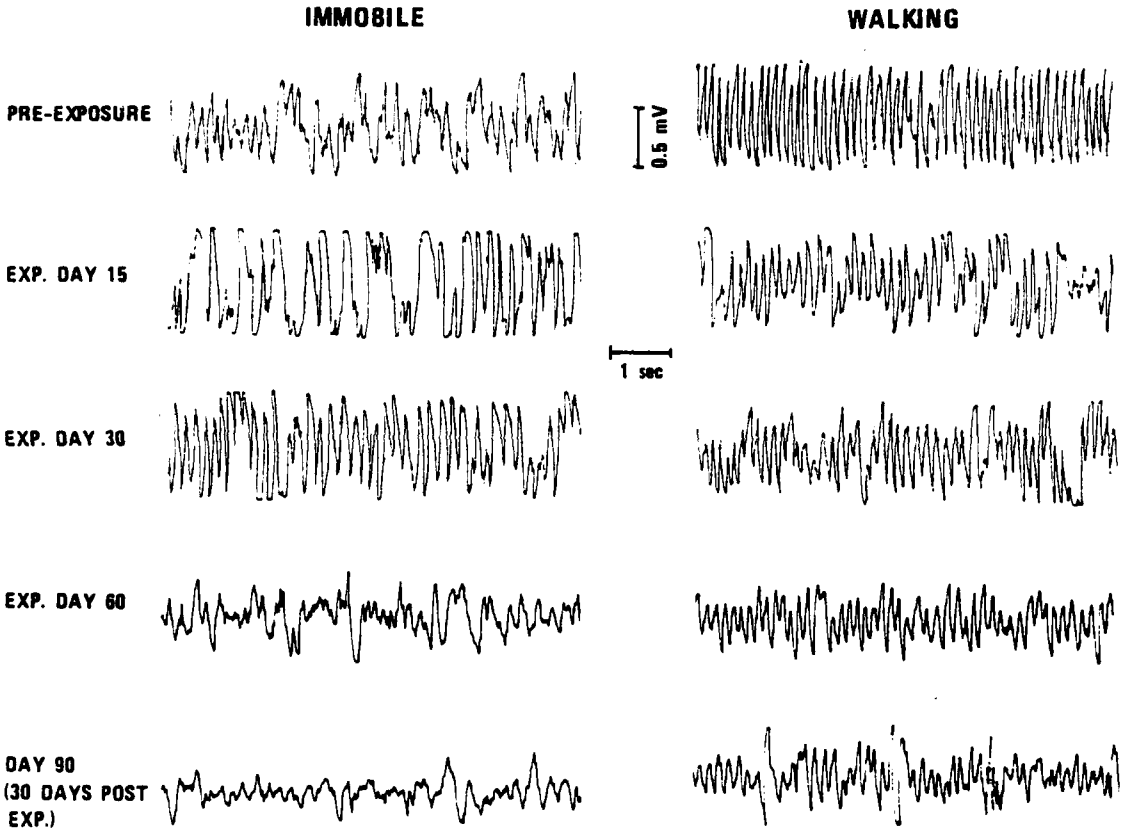


FIGURE 4.

Electric activity in the dorsal hippocampal formation before, during and after a 60 day period of toluene inhalation, 500 ppm for sixteen hours per day, five days per week. Days without exposure are not counted in the exposure period. The records are from bipolar registrations from a single rat.



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